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Award Number: W81XWH-07-1-0120

TITLE: The Role of YY1 in Prostate Cancer

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REPORT DATE: March 2010

TYPE OF REPORT: Final

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

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REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

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1. REPORT DATE *(DD-MM-YYYY)* 31/03/2010	2. REPORT TYPE Final	3. DATES COVERED *(From - To)* 03/01/2007 – 02/28/2010

4. TITLE AND SUBTITLE

The Role of YY1 in Prostate Cancer

5a. CONTRACT NUMBER
W81XWH-07-1-0120

5b. GRANT NUMBER
PC060490

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

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5d. PROJECT NUMBER
W91ZSQ6287N656

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Wake Forest University School
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Medical Center Boulevard
Winston-Salem, NC 27157-0001

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for public release; distribution unlimited

13. SUPPLEMENTARY NOTES
YY1, Ezh2, p53, Mdm2, Prostate Cancer, Lentivirus, siRNA, knockdown, overexpression, renalgraft, mouse model.

14. ABSTRACT

Our previous studies showed that CMV promoter-driven YY1 overexpression did not form any tumor in renalgraft studies. To repeat this experiment alternatively, we made a new vector with chicken-beta actin promoter and IVS(II) that support sustained and high YY1 expression. Our previous studies also showed that Pten-null prostate cells with siRNAs did not form any graft. We believe the cells have lost their stem/progenitor cell features. We thus made new batches of Pten-null clones that formed tumorigenic grafts in mice. We determined that residues 201-226 of YY1 interact with both Hdm2 and Ezh2. To distinguish YY1's regulation to these two proteins, we generated 5 YY1 mutants with alanines replacing the residues in this region. We compared the Hdm2- and Ezh2-interactions of wild type YY1 and these mutants. The preliminary results indicated that all these mutants lost their binding affinity to Hmd2, while some retained different degrees of interactions with Ezh2. We also observed the interaction of YY1 and Pten, which could be disrupted by p53, suggesting Pten and p53 compete in binding to YY1. Interestingly, YY1 overexpression negatively regulates the endogenous levels of Pten, suggesting an oncogenic or proliferative activity of YY1.

15. SUBJECT TERMS
None provided.

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON USAMRMC
a. REPORT U	**b. ABSTRACT** U	**c. THIS PAGE** U	UU	49	**19b. TELEPHONE NUMBER** *(include area code)*

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

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A. Introduction

Prostate cancer (PCa) is a major public health problem among men of many countries. In the United States, one of six men will be diagnosed with prostate carcinoma in their life time and PCa is the second leading cause of cancer-related deaths. The causes that lead to the development of PCa include genetic changes, such as mutations of tumor suppressor genes, and aberrant epigenetic alterations, defined as heritable changes in gene expression without changes in DNA sequence, contribute to prostate malignancy [1].

As a multifunctional protein, Yin Yang 1 (YY1) [2] is overexpressed in several types of cancers, including PCa [3-6]. YY1 is a multifunctional protein that acts as a transcription factor and plays a crucial role in epigenetic regulation, including mediating histone acetylation, deacetylation and methylation [7-9]. Data from our laboratory and others indicated that YY1 is overexpressed in both human PCa [3] and the PCa of a transgenic mouse model. The goal of this project is to determine the functional role of overexpressed YY1 in PCa. Our overall hypothesis is that overexpressed YY1 is essential to the development and progression of PCa. We will perform in vitro and in vivo studies to test this hypothesis. In addition, we will also investigate how the interplay of YY1-p53-Pten and YY1-Ezh2 contributes to the tumor development of PCa. Our study will improve the understanding of the mechanism that leads to the aberrant epigenetic regulation in PCa. This will provide fundamental support to the development of therapeutic approaches to conquer PCa by reversing its epigenetic abnormality. Since YY1 is overexpressed in multiple tumors, the results may also be applied to other types of cancers. Especially, if YY1 can be proved as a therapeutic target in cancer therapy to reverse the tumorigenic properties of cancers, it will be particularly helpful to treat these cancers that surgical approaches cannot be applied.

B. Body:

Task 1. To generate Lentiviruses expressing YY1 or different siRNAs (Months 1-8):
    a. Make *yy1* siRNA construct that attenuates overexpressed YY1 of Pten$^{-/-}$ MPECs to a level comparable to that of Pten$^{+/+}$ MPECs (Month 1-3).
    b. Make YY1 expression constructs that express YY1 in Pten$^{+/+}$ MPECs at a level that is about 5 folds higher than the endogenous YY1 (Month 1-3).
    c. Transfer the expression cassettes of YY1 and *yy1* siRNA to the Lentivirus vector (LTV-1). Produce the viruses and determine their titers (Month 4-5).
    d. Examine the YY1 knocking down and YY1 overexpression in the cultured MPECs. Generate stable MPECs expressing *yy1* siRNA, scrambled siRNA, or YY1 (Month 6-8).

Task 1 has been fulfilled. See the annual report in March 2008.

Task 2. To study the effects of YY1 expression to the PCa formation *in vivo* (Months 9-17):
    a. Start the PCa tumor formation experiments in mice by renal grafting (1) Pten$^{-/-}$ MPECs expressing the scrambled siRNA and *yy1* siRNA; (2) Pten$^{+/+}$ and Pten$^{+/-}$ MPECs containing the YY1 expression cassette and the empty vector (Months 9-12).
    b. Data analysis of the *in vivo* study, including dissection of the graft, histopathological study, immunohistochemistry and the assessment of the results (Months 13-15).
    c. Determine whether YY1 overexpression in PCa is regulated at the transcriptional or posttranslational level (Months 16-17).

Task 2 has been performed. See the annual report in March 2009.

Brief summary and additional data:
1. As we showed in the annual reports of March 2008 and March 2009, we could successfully knock down YY1 in cultured Pten$^{-/-}$ MPECs (mouse epithelial prostate cells). These cells with YY1 knockdown exhibited decreased proliferation in vitro. However, in the renalgraft study, these Pten$^{-/-}$ MPECs infected by lentiviruses expressing YY1 siRNA and scrambled siRNA did not form any renalgraft with meaningful sizes (see Figure 3 in Annual Report 2009). We predicted that the following reasons could lead to this unexpected result: (1) the MPECs might have lost their properties as stem/progenitor cells after multiple passages; (2) the isolated urogenital mesenchyme (UGM) might not be functional due to in vitro culture process following a published protocol from Dr. Witte group [10]. Further study helped us exclude the possibility (2), since the repeated renalgraft experiment with freshly isolated UGM still could not form good renalgrafts. Therefore, we decided to re-make the Pten$^{-/-}$ MPECs by following the procedure below:

1) Isolate a new batch of Pten$^{LoxP/LoxP}$ prostate cells from Pten$^{LoxP/LoxP}$ mouse prostate and make single clones by limited-dilution;
2) Use renalgraft studies to test the stem/progenitor properties of these isolated clones and select the clones that form prostate-like tissues under renal capsule [11];
3) Individually transfect these clones with pCMV/Cre + EGFP and use FACS to isolate the EGFP-positive cells/clones;

4) Expand the cells of these clones and carry out Western blot to determine the Pten status in these clones to get Pten$^{-/-}$ MPEC lines;
5) Study the tumor formation of these Pten$^{-/-}$ MPEC clones using the standard renalgraft experiments [11];

As a result of these studies, we were able to obtain a number of Pten$^{-/-}$ MPEC clones (see Figure 1A for a representative Western blot study). We observed that several of these Pten$^{-/-}$ clones could form prostate-like tumors (see Clones 1, 2 and 7 and compare them with Clone A in Figure 1B). We are currently using Clone 2 to knock down YY1 using both constitutive siRNA vector and a Tet-On inducible vector. The generated cells will be tested in vitro first for YY1 expression and cell proliferation, and then used to study the effects of YY1 depletion on prostate cancer formation in vivo.

2. We also observed that previous MPECs could not maintain decreased YY1 expression after we infected them by lentiviruses expressing YY1 siRNA. As a result, in 1~2 months, the YY1 expression in these cells could be restored. This may affect the result of our renalgraft experiment (2~3-month growth of renalgrafts in vivo) to study

Figure 1. **A.** Western blot to confirm the Pten deletion in screened clones. Clone A: Pten$^{LoxP/LoxP}$ MPECs; Clone 1-5: examples of screened clones. **B.** A representative renalgraft study of selected clones. Clone A forms normal prostate-like tissues, while the Pten$^{-/-}$ clones (clones 1, 2 and 7) derived from Clone A by Cre-transfection form prostate tumor-like tissues.

the effect of YY1 knockdown in MPECs. Therefore, we are currently also generating a Tet-On inducible siRNA system to knock down YY1.

3. YY1 overexpression: In the annual report of March 2009, we described that YY1 overexpression driven by CMV promoter in pSL2 lentiviral vector did not make the MPECs change the morphology in renalgraft studies when compared with the control renalgrafts produced by the MPECs infected by pSL2 vector. It was possible that these MPECs lost increased YY1 expression in these renalgrafts, since the CMV promoter in pSL2 vector could be inactivated in vivo epigenetically [12]. We indicated that we would construct a new vector pSL5 that employs chicken β-actin promoter (cβ-actin P) [13], instead of the CMV promoter. The cβ-actin P sustains stable expression of ectopic genes, although the expression levels are not as high as that of the CMV promoter. We have therefore generated the pSL5/YY1 vector and tested the YY1 expression levels. However, we found that YY1 increase was very modest (2~3-fold over the endogenous YY1). To further increase YY1 expression, we inserted an IVS(II) sequence between the cβ-actin P and YY1 cDNA. The IVS(II) is derived from rabbit β-globin intron II and has been reported to significantly enhance the expression of its downstream cDNAs [14]. Indeed, we observed that the pSL5-
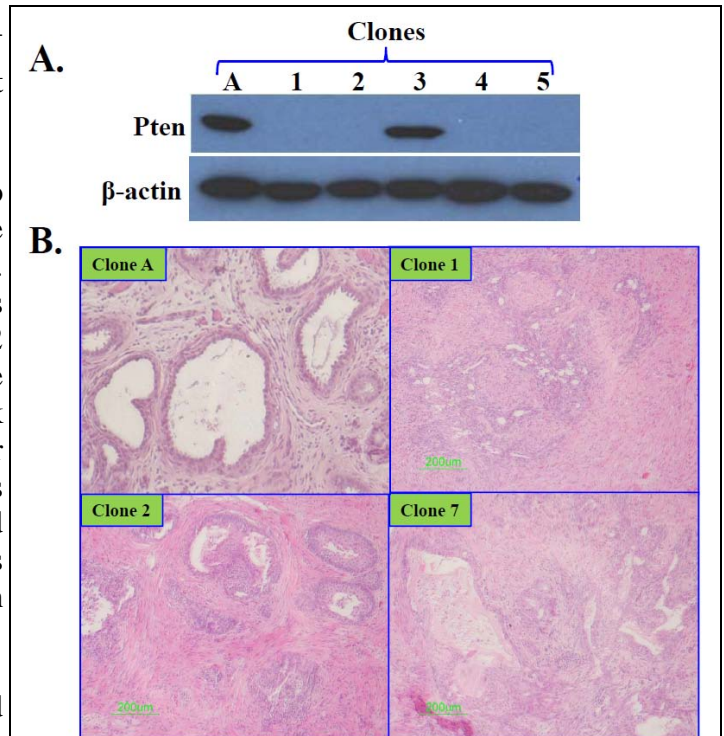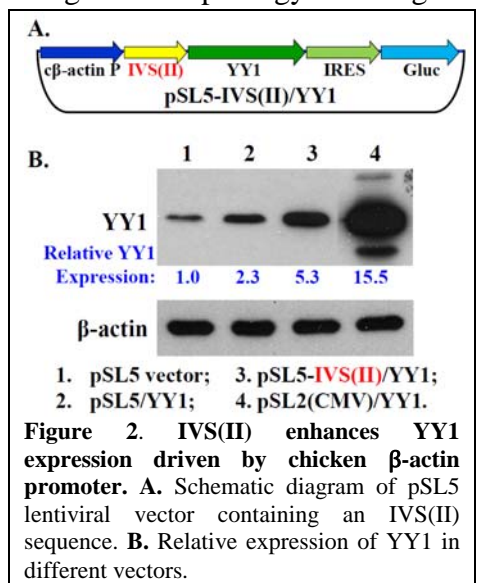

Figure 2. **IVS(II) enhances YY1 expression driven by chicken β-actin promoter. A.** Schematic diagram of pSL5 lentiviral vector containing an IVS(II) sequence. **B.** Relative expression of YY1 in different vectors.

IVS(II)-YY1 (Figure 2A) increased YY1 expression from 2.3-fold of pSL5/YY1 to the 5.3-fold of pSL5-IVS(II)/YY1 (Figure 2B, compare lanes 3 and 2 with 1). Although this expression by pSL5-IVS(II)/YY1 is not as high as the over 15-fold increase by pSL2/YY1 (with the CMV promoter), it did not show these extra bands that were generated due to the robust YY1 expression (Figure 2B, compare lanes 3 and 4) and should be more likely to recapitulate the conditions of YY1 increase in physiological prostate cancer.

Currently, we are generating stable MPEC lines infected by pSL-IVS(II)/YY1 virus and the vector control. After confirming their stable YY1 expression and testing the cell proliferation in vivo, we will use these cells for renalgraft studies.

4. When the renalgraft study mentioned above is carried out with these Pten$^{-/-}$ cells, we will analyze the correlation between tumor grades and YY1 mRNA/protein levels to determine whether YY1 overexpression is due to upregulation of YY1 gene or increased stability of YY1 protein.

Task 3. To study the tumor formation with disrupted YY1-p53 interaction (Months 18-26):
  a. Produce Lentivirus containing the vector or the expression cassette for the YY1 mutant deficient in mediating p53 degradation. Use the Lentivirus to generate sable Pten$^{-/-}$ MPECs carrying the vector or expressing this YY1 mutant (Months 18-19).
  b. Start the *in vivo* tumor formation experiment by renal grafting these MPECs (Months 20-22).
  c. Data analysis of the *in vivo* study, including the dissection of the renal graft, histopathological study, immunohistochemistry and the assessment of the results (Months 23-24).
  d. Determine if the interactions of YY1-p53 and YY1-Pten are exclusive (Months 25-26).

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1. The study of YY1 mutant deficient in mediating p53 degradation will need the Pten$^{-/-}$ cell lines described in Figure 1. These cell lines (particularly the Clone 2) are currently tested for YY1-siRNA mediated knockdown and renalgraft studies. Once this is completed, we will then use YY1 mutant(s) deficient in mediating p53 ubiquitination/degradation to replace the endogenous YY1. The renalgraft study and data analysis will then be followed.

2. In the annual report of March 2009, we described that we had narrowed down the region of residues responsible for interacting Hdm2 (human Mdm2) from our previously reported residues 201-295 (see [15]) to 201-226 of YY1. This is the region mediating Hdm2 function and therefore regulating p53 degradation. However, this region coincidentally overlaps with the REPO domain of YY1 that recruits Ezh2 and other polycomb group proteins [16] to YY1 target promoters. Therefore, it is necessary to further map this region in order to distinguish the regulation of YY1 to these two pathways of Mdm2 and Ezh2.

We first employed "alanine-scan" to make 5 YY1 mutants with each 5 or 6 residues in the amino acids 201-226 of YY1 replaced by 5 or 6 alanines, respectively (Figure 3). We subcloned these 5 YY1 mutants, as well as wild type (wt) YY1, YY1-ΔREPO and YY1Δ(227-295) into a pcDNA/3×Flag vector and studied the interaction of these mutants with Mdm2 and Ezh2 (see Task 4) using the co-immunoprecipitation (Co-IP) experiments. To study their interaction with Hdm2, these YY1 expression vectors were individually cotransfected with pCMV/Hdm2 into HeLa cells. After 48 h, cell lysates were treated by Flag antibody-conjugated

REPO domain of YY1

	201	206	211	216	221	226
wild type (wt)	GNKKW	EQKQV	QIKTL	EGEFS	VTMWS	S
Mut 1: (201-205) →5A	AAAAA	EQKQV	QIKTL	EGEFS	VTMWS	S
Mut 2: (206-210) →5A	GNKKW	AAAAA	QIKTL	EGEFS	VTMWS	S
Mut 3: (211-215) →5A	GNKKW	EQKQV	AAAAA	EGEFS	VTMWS	S
Mut 4: (216-220) →5A	GNKKW	EQKQV	QIKTL	AAAAA	VTMWS	S
Mut 5: (221-226) →6A	GNKKW	EQKQV	QIKTL	EGEFS	AAAAA	A

Figure 3. The sequences between residues 201-226 of YY1 wt and the generated five mutants for "alanine scan" to further map the function of REPO domain.

agarose beads and the proteins brought down were analyzed by Western blot. As shown in Figure 4, YY1 wt and Δ227-295 could pull down significant amounts of Hdm2, while none of the other YY1 mutants could bring down any detectable Hdm2. The results suggest that the whole region of 201-226 is essential to the interaction of YY1 with Hdm2. We have noticed that the expression levels of some YY1 mutants were not even (middle panel of Figure 4). Therefore, we are currently repeating this experiment to confirm this result.

3. To determine the interaction of YY1-p53 and YY1-Pten:

3.1. We first carried out protein binding experiments to determine the interaction between YY1 and Pten. As shown in Figure 5A, GST-Pten, but not GST alone, could bring down His-YY1 in an *in vitro* binding study using proteins expressed and purified from bacteria (compare lanes 4 and 5). We used GST-p53 (lane 3) as a positive control since it can interact with YY1 [15].

In a reciprocal protein binding experiment (Figure 5B), we observed that GST-YY1, instead of GST alone, could pull down His-Pten (compare lanes 4 and 5). In this case, we used GST-p53 as a positive control (lane 3), since Pten-p53 interaction has been previously reported [17].

We also carried out Co-IP experiments by transfecting pcDNA3/Flag-Pten and pcDNA3/HA-YY1. We observed that Flag antibody, but not the control antibody, could bring down HA-YY1 only when these two plasmids were cotransfected (compare lanes 4 and 5 with 2 and 3, Figure 5C). Reciprocally, YY1 antibody, but not the control antibody, could pull down Flag-Pten only in the cells transfected by these two plasmids (compare lanes 4 and 5 with 2 and 3, Figure 5D). Importantly, in the cotransfection of HA-YY1 and Flag-Pten, when pCMV/p53 was cotransfected, YY1-Pten interaction was attenuated (compare lanes 6 and 7 with 4 and 5, Figure 5C). This suggests that Pten and p53 competitively interact with YY1 and their binding sites on YY1 may overlap.
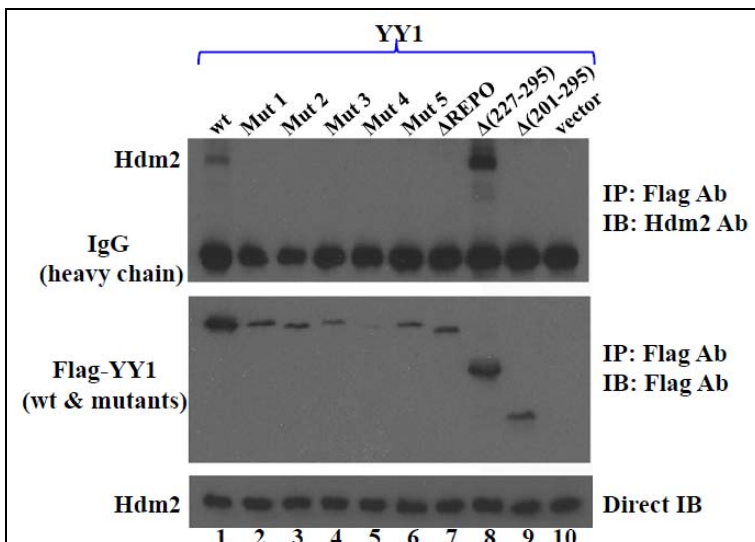

Figure 4. Co-IP studies to further map the binding domain(s) of YY1 and Hdm2. The mutants 1-5 are indicated in Figure 3. IP: immunoprecipitation; IB: immune blot; Ab: antibody.
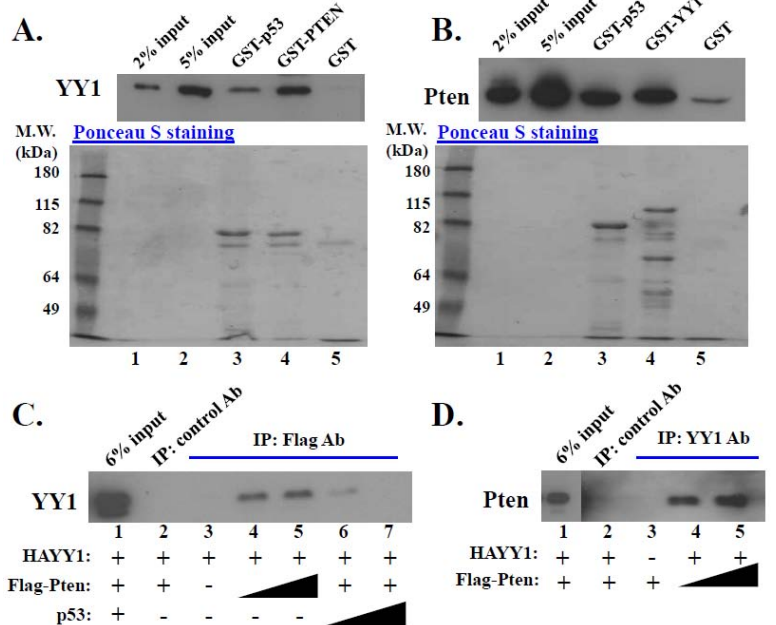

Figure 5. In vitro (**A, B**) and in cell (**C, D**) interaction of YY1 and Pten, and the effects of p53 on YY1-Pten binding (**C**).
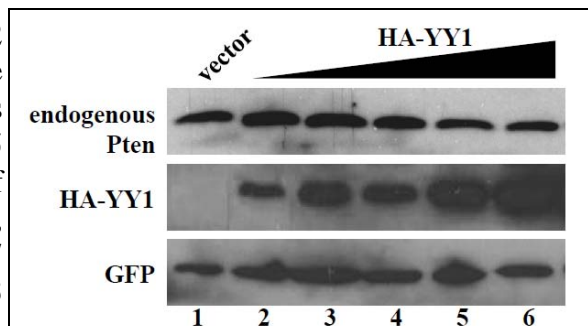

Figure 6. Effects of YY1 overexpression on endogenous Pten expression.

3.2. We also studied the effects of YY1 overexpression on endogenous Pten levels. Increasing amounts of pcDNA3/HA-YY1 and equal amount of pCMV/EGFP (a transfection control) were transfected to 293T cells. After 48 h, cell lysates were analyzed by Western blot using Pten, HA and GFP antibodies. When HA-YY1 was overexpressed, we observed a gradual decrease of expression of endogenous Pten (Figure 6), suggesting that YY1 may antagonize the expression and/or promote the degradation of Pten.

Task 4. To study the tumor formation with disrupted YY1-Ezh2 association (Months 27-36):
   a. Generate an YY1 mutant that lacks the ability of Ezh2 association. Produce Lentiviruses that contain the vector or the expression cassette of this YY1 mutant. Use the Lentiviruses to generate sable Pten$^{-/-}$ MPECs carrying the vector or expressing this YY1 mutant (Months 27-28)
   b. Start the tumor formation experiment in mice by renal grafting these MPECs (Months 29-32)
   c. Data analysis of the *in vivo* study, including dissection of the renal graft, histopathological study, immunohistochemistry and the assessment of the results (Months 33-34)
   d. Determine if elevated YY1 has any regulatory effect to the Ezh2 overexpression in PCa (Months 35-36).

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1. We are facing the same dilemma as Task 3 waiting for the further characterization of the new Pten$^{-/-}$ clones described in Task 2 before conducting the functional study of YY1-Ezh2 interaction in prostate cancer formation. Most parts of this Task will be followed after the renalgraft studies are carried out.

2. As stated in Task 3, the domains of YY1 responsible for interacting with Mdm2 and Ezh2 overlap among the residues of 201-226 (REPO domain [16]). Therefore, we are also attempting to further study the REPO domain of YY1 to determine the residue(s) responsible for binding to Ezh2.

As shown in Figure 3, we have generated 5 YY1 mutants (mutants 1 - 5) with every 5 or 6 residues in 201-226 replaced by 5 or 6 alainines. We therefore studied the interaction of these YY1 mutants with Ezh2. Flag-tagged YY1 wt and mutants (as labeled on the top of Figure 7) were individually cotransfected with pcDNA3/HA-Ezh2. After 48 h, the cell lysates were treated with Flag antibody-conjugated agarose beads, followed by Western blot studies using HA and Flag antibodies. As shown in the top panel of Figure 7, while YY1Δ(227-295) retained the binding affinity to Ezh2, all other YY1 mutants exhibited decreased interaction with Ezh2. Especially, mutant 1 showed nearly totally lost Ezh2-binding, as YY1ΔREPO did [16]. Meanwhile, some of the YY1 mutants, especially mutant 3, retained significant levels of affinity to Ezh2.
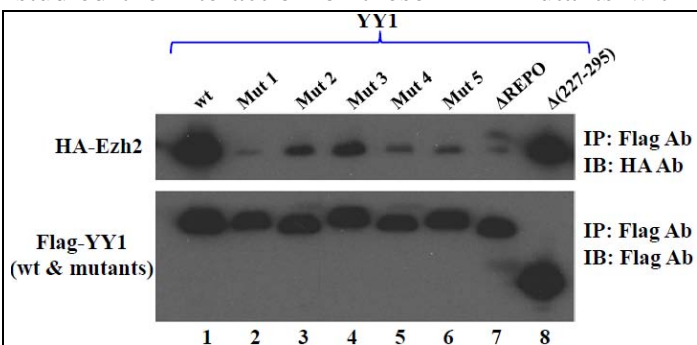

Figure 7. Co-IP studies to further map the binding domain(s) of YY1 and Ezh2. The mutants 1-5 are indicated in Figure 3.

We are repeating these Co-IP experiments to narrow down a smaller region or residue(s) responsible for Ezh2 interaction. Such YY1 mutant(s) will be used to study the functional role of YY1-Ezh2 interaction in prostate cancer formation by Pten$^{-/-}$ MPECs using the renalgraft experiment.

C. Key Research Accomplishments:
In addition to the "Key Research Accomplishments" in the annual reports of 2008 and 2009, we have the following new accomplishments in the last year of this award:

1. We have generated a new lentiviral vector that contains chicken β-actin promoter and sustain long-term ectopic gene expression *in vitro* and *in vivo*. Especially, the insertion of IVS(II) sequence between the chicken β-actin promoter and YY1 cDNA significantly enhanced YY1 expression.

2. We started to further map the residues 201-226 of YY1 to (1) distinguish the interaction of YY1 with Hdm2 and Ezh2, and (2) to marrow down the residues of YY1 that are responsible for interacting with these two proteins. Initial results indicate that the integrity of this region is essential to the interaction of YY1 with Hdm2 and Ezh2.

3. We detect the direct interaction of YY1 and Pten using both purified proteins and the Co-IP experiments. Further mapping studies will be needed to determine the specific interaction domains on these two proteins.

4. We observed that YY1-Pten interaction is competitively attenuated by p53 in a cell-based Co-IP study. This suggests that the interaction sites of Pten and p53 on YY1 may overlap. We also detected that YY1 overexpression attenuated the expression of endogenous Pten.

D. Reportable Outcomes:
In addition to the "Reportable Outcomes" in the annual reports of 2008 and 2009, we have the following new outcomes in the last year of this award:

1. Since the previous $Pten^{-/-}$ MPECs lost its properties of progenitor/stem cells, we re-generated $Pten^{-/-}$ cell lines and determined their properties of forming prostate cancer in renalgraft studies. These cell lines will be used to repeat the study for the functional role of YY1 knockdown in prostate cancer development.

2. The disruption/mutation by alanine-scan in the region of 201-226 of YY1 leads to the functional loss of YY1 in its interaction with Hdm2 and Ezh2.

3. YY1 directly interacts with Pten and negatively regulates endogenous Pten expression, which is reminiscent of the negative regulation of YY1 to p53. However, Pten and p53 competitively interact wit YY1, suggesting that YY1 may regulate these two tumor suppressors differentially.

4. YY1 overexpression itself is unlikely to induce any tumor formation *in vivo*, based on the renalgraft experiments using lentivirus produced by pSL2-YY1 (with CMV promoter). However, we are still attempting to use a vector containing chicken β-actin promoter and an IVS(II) sequence to repeat this experiment.

E. Conclusion:
In addition to the "Conclusion" in the annual reports of 2008 and 2009, we have the following additional conclusion based on the studies in the last year of this award:

1. We observed that YY1 overexpression driven by CMV promoter is insufficient to induce tumor formation in the renalgraft experiment. We are attempting to use an alternative approach to express YY1 and repeat the experiment.

2. We observed that Pten$^{-/-}$ MPECs expressing YY1 siRNA and scrambled control siRNA did not successfully form renalgrafts *in vivo*. Our further investigation indicated that these Pten$^{-/-}$ cells may have changed or lost their characterized features due to multiple passages. We have re-generated new batches of Pten$^{-/-}$ MPECs and determined their tumor formation properties *in vivo*. These cells will be used in the studies for the functional role of YY1 depletion in prostate cancer formation.

3. The studies for the functional roles of YY1-Hdm2-p53 and YY1-Ezh2 interactions in prostate cancer development could not be carried out due to the technical difficulties of using Pten$^{-/-}$ MPECs. Once these new Pten$^{-/-}$ MPECs have been well characterized, we will use these cells for these studies.

4. While the studies for the functional roles of YY1-Hdm2-p53 and YY1-Ezh2 interactions wait for the characterization of Pten$^{-/-}$ MPECs, we have put great efforts in the molecular biology studies to further map the interaction domains of Hdm2 and Ezh2 on YY1 protein. We determined that these two proteins interact to the same region of YY1 (residues 201-226). We are now further mapping this region to distinguish YY1's interactions with Hdm2 and Ezh2. The new mutants have been generated and well expressed. Preliminary data of the interaction domain mapping have been obtained and will be repeated.

5. In addition to our previous discovery of YY1's negative regulation to p53, we also observed that YY1 overexpression downregulates endogenous Pten expression. This observation again suggests the oncogenic or proliferative properties of YY1 in cancer development, as we previously published.

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G. Appendices:

1. List of personnel supported for meeting:

(1) Guangchao Sui
Meeting title: Cancer Epigenetics,
Date: May 28 - 31, 2008
City: Boston Park Plaza Hotel, Boston, Massachusetts

Abstract
Title: Modulation of androgen receptor transcriptional activity by YY1

Authors: Zhiyong Deng*, Meimei Wan, Paul Cao, Scott D. Cramer, Guangchao Sui
Department of Cancer Biology and Comprehensive Cancer Center, Wake Forest University School of Medicine, Winston-Salem, NC 27157
* Presenter

Abstract: Prostate cancer is one of the most common forms of cancer among men and also the second leading cause of cancer-related death in the United States. Development of prostate cancer is characterized by aberrant epigenetic changes, including DNA hypo- or hypermethylation and altered histone modification. Previous investigations have shown that androgen receptor (AR) plays an important role in prostate cancer development. As a hormone receptor and a transcription factor, AR is involved in different signaling pathways and facilitates prostate cancer development and progression by regulating its target gene expression. Transcriptional activity of AR is also modulated by a variety of proteins, including CBP/P300 and LSD1. In this study, we investigated the functional interplay between Yin Yang 1 (YY1) and AR. YY1 is a multifunctional protein implicated in epigenetic regulation of gene expression and protein posttranslational modification. We observed that the association of endogenous AR and YY1 in LNCaP cells in immunoprecipitation study. Our in vitro protein binding experiments also demonstrated that YY1 directly bound to the C-terminus of AR where its DNA and ligand binding domains are located. In addition, the zinc finger domain of YY1 was found to be necessary for its capability of binding to AR. Using the 5.8kb-PSA promoter-Luciferase as a reporter, we found that overexpression of YY1 enhanced the transcriptional activity of AR, and conversely knocking down of YY1 by shRNA led to deceased transcriptional activity of AR. Further studies indicated that YY1-AR protein interaction is important for YY1-mediated AR function, since an YY1 chimeric mutant that lacks the binding affinity to AR lost its regulation to the transcriptional activity of AR. In conclusion, YY1 positively regulates the transcriptional activity of AR and this regulation is dependent on the physical interaction of these two proteins.

(2) Guangchao Sui
Meeting titles: BACR/EACR Symposium – Chromatin and Cancer Meeting; Transcription and Cancer Meeting
Date: July 6 - 8, 2009; July 8 - 10, 2009;
City: University of Cambridge, Cambridge, UK

Abstract (1)
Title: Yin Yang 1 Promotes Prostate Cell Proliferation and Is Essential to Prostate Cancer Development

Author: Meimei Wan*, Weiwei Huang, Linara Axanova, Paul Cao, Scott Cramer, Yong Q. Chen and Guangchao Sui
* Presenter

Department of Cancer Biology and Comprehensive Cancer Center, Wake Forest University School of Medicine, Winston-Salem, NC 27157

Yin Yang 1 (YY1) plays an essential role in regulating chromatin remodeling. YY1 interacts with various histone modifiers, such as HDAC1-3, p300, Ezh2 and PRMT1, and consequently mediates histone modifications on its target promoters. YY1 is overexpressed in various types of cancers, including prostate cancer. The histone methyltransferase Ezh2, which has been indicated to play an essential role in prostate cancer progression and invasiveness, depends on the recruitment of YY1 to exert its function. Our recent study also revealed that YY1 enhances p53 degradation and this function is independent of the transcriptional activity of YY1. In this study, we demonstrated that YY1 expression is significantly elevated in a prostate cancer mouse model with prostate-specific Pten knockout. Using clonogenic assay, a three-dimensional collagen culture system and mouse xenograft experiments, we studied the effects of YY1 depletion and increase on prostate cell proliferation and tumor formation. We report here that YY1 knockdown in isolated mouse prostate epithelial cells (mPECs) with tumorigenic potential decreases the cell proliferation, survivability and xenograft tumor formation. Conversely, ectopic expression of YY1 in normal mPECs promoted cell proliferation in vitro. Overall, our study demonstrates that YY1 expression positively correlates with prostate cell proliferation and suggests an etiological role of YY1 in prostate cancer development. We are currently investigating the functional domains of YY1 that mediate the degradation of p53 and the methyltransferase activity of Ezh2, and studying the effects of altered YY1 expression on prostate cancer formation in a renalgraft mouse model.

Abstract (2)
Title: The Potential of Yin Yang 1 as a Biomarker Gene and Therapeutic Target of Breast Cancer
Authors: Meimei Wan*, Weiwei Huang, Paul Cao, Zhiyong Deng, Gregory B. Russell, Timothy E. Kute, Steven A. Akman and Guangchao Sui
* Presenter

Department of Cancer Biology and Comprehensive Cancer Center, Wake Forest University School of Medicine, Winston-Salem, NC 27157

As a multifunctional transcription factor, Yin Yang 1 (YY1) regulates a myriad of cancer-related genes, such as c-myc, c-fos, p53 and ERBB2. Meanwhile, increased YY1 expression has been observed in various types of cancers, including breast cancer. However, a causal link has not been established between YY1 increase and breast cell tumorigenesis. In this study, we evaluated YY1 expression in a breast cancer tissue microarray (TMA) with 120 patient samples and confirmed that YY1 is significantly overexpressed in human breast cancer compared to normal breast tissues. Interestingly, YY1 increases are associated with extended reoccurrence time among the breast cancer patients. Consistently, we also observed that YY1 levels in 5 different breast cancer cell lines are elevated by >2-fold compared to non-tumorigenic MCF-10A cells and >5-fold compared to normal human breast mammary cells. Knocking down of YY1 inhibited MCF-7 cell growth, but did not exhibit significant effect on MCF-10A cells. Importantly, altering YY1 expression could exchange the morphological features between MCF-7 cells and MCF-10A cells in both regular culture dishes and three-dimensional Matrigel culture system. Thus, our study demonstrates that YY1 increase can potentially be used as a biomarker in breast cancer diagnosis and YY1 is a promising therapeutic target to reverse the aberrant epigenetics of breast cancer and consequently cure this important disease.

2. List of published papers and manuscripts:

   (1) Guangchao Sui. The Regulation of YY1 in Tumorigenesis and its Targeting Potential in Cancer Therapy, Mol Cell Pharmacol 2009;1(3):157-176.

   (2) Zhiyong Deng, Paul Cao, Meimei Wan and Guangchao Sui. Yin Yang 1: a multifaceted protein beyond a transcription factor. Transcription 2010 (submitted).

3. Curriculum vitae of Guangchao Sui.

The Regulation of YY1 in Tumorigenesis and its Targeting Potential in Cancer Therapy

Guangchao Sui

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Abstract

Yin Yang 1 (YY1) is a multifunctional protein and regulates various processes of development and differentiation. Increasing evidence indicates an essential role of YY1 in tumorigenesis. As a transcription factor, YY1 regulates the expression of numerous genes that are mostly involved in cancers. YY1 can either activate or repress the target genes, depending on the cofactors that it recruits. Importantly, most studies to date suggest a proliferative or oncogenic role of YY1 in cancer development. Meanwhile, overexpression of YY1 has been observed in different types of cancers and YY1 has been proposed as a potential prognostic marker of these cancers. A reasonable hypothesis is that upregulated YY1 leads to unbalanced expression of its target genes and in turn initiates or arguments tumorigenesis. Ample studies indicate that YY1 exerts broad regulation in various epigenetic events, especially histone acetylation and methylation. Since most cancers exhibit deregulated epigenetics, overexpressed YY1 may contribute to these aberrant epigenetic statuses in cancer cells. The epigenetic processes regulated by YY1 are reversible. Therefore, it is possible that targeting YY1 may adjust various deregulated epigenetic events in cancer cells, restore the normal epigenetic conditions and consequently block cancer development. This review summarizes cancer-related studies of YY1 and discusses the potential of YY1 as a target of cancer therapy.

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1. Introduction

Aberrant expression and function of transcription factors play vital roles in oncogenic transformation of cells in different tissues. These factors interact with specific DNA elements and other protein cofactors to form transcriptional machinery that can either activate or repress the expression of essential genes involved in tumorigenesis. The altered or enhanced functions of these factors contribute, either directly or indirectly through other downstream pathways, to some or all of the cancer hallmarks, including insensitivity to antigrowth or apoptotic signals, self-sufficient growth signals, sustained angiogenesis, limitless replicative potential and invasive or metastatic capability (1).

Yin Yang 1 (YY1) is a ubiquitously expressed protein in all tissues and highly conserved in many different species. YY1-related studies keep accumulating and have branched to almost all existing research areas with over 700 papers available to date. Therefore, it is unrealistic to embrace all of these publications and even every research field in this review. For a comprehensive understanding of YY1 gene, protein and its regulatory models, please refer to the two excellent review articles (2,3) from the pioneers of YY1 studies, Drs. Shi and Seto. Some other reviews also discussed the role of YY1 in cancer development (4-6). The focus of this article is the cancer-related function and regulation of YY1. The potential of YY1 as a therapeutic target of cancer therapy is also discussed.

1.1. Discovery of YY1

YY1 was initially discovered in 1991 by Shi et al as a transcriptional factor binding to the P5 promoter of adeno-associated virus (7). The inhibitory function of YY1 to this promoter can be converted to an activating effect upon association with a viral protein, E1A. By its very name, "Yin

Yang" represents its two opposite capabilities of either repressing or stimulating gene transcription, and both functions have been demonstrated in numerous studies. In addition, YY1 can act as an initiator to direct and activate gene transcription (8). Two subsequent studies also reported subcloning of YY1 cDNA (NF-E1 and delta, respectively) and observed the regulatory role of YY1 in gene transcription. One of the studies demonstrated that YY1 is a binding protein of the negative-acting segment of the immunoglobulin kappa E3' enhancer (9). The other study showed that YY1 acts as a transcription activator of two genes coding ribosomal proteins rpL30 and rpL32, since the deletion of YY1 binding elements dramatically reduced their expression (10). In 1992, YY1 (named as UCRBP) was identified as a binding protein of the upstream conserved region in Moloney murine leukemia virus (MuLV) and negatively regulated the MuLV promoter activity (11).

1.2. Expression and general function of YY1

YY1 is ubiquitously expressed in all tissues and highly conserved among different species. Currently, YY1 cDNAs have been cloned from many species, including human (7), mouse (10,12), rat (13), chicken (14), zebrafish (15) and xenopus (16). Drosophila has two orthologs of YY1, pleiohomeotic (*pho*) and *pho* like (*phol*) (17,18), which have high degrees of similarity in the zinc finger regions with those of human YY1. The Seto group discovered a protein called YY2 that has 65% similarity in DNA sequence and 56% similarity in protein sequence to human YY1 (19). Due to the pronounced similarity at the zinc finger regions of these two proteins, YY2 binds to the same consensus sequence as YY1 but with much lower affinity (20). A latter report suggested that YY2 is a retroposed copy of YY1 that has been inserted into another gene locus (21).

A number of studies demonstrated the essential roles of YY1 in normal biological processes, such as embryonic development and gene imprinting. Donohoe et al attempted to generate YY1 knockout mice, but discovered that YY1 deletion is lethal to mouse embryonic development (22). The complex of YY1 and a chromatin isolator, CTCF, regulates the X chromosome binary switch by binding to a non-coding locus, Tsix that controls chromosome pairing and counting (23). Meanwhile, YY1 has been shown as a critical regulator of early B-cell development (24), genomic imprinting (25), and neuron development and differentiation (26,27).

Affar et al presented a comprehensive study on the essential dosage-dependent functions of YY1 in embryonic development and cell cycle progression (28). They provided clear evidence that decreases of YY1 impair the growth and viability of mouse embryos in a dose-dependent manner. In addition, levels of YY1 tightly correlate with cell proliferation, and its depletion prevents cytokinesis and increases cellular sensitivity to various apoptotic stimuli.

2. YY1 as a transcription factor

The models of YY1-mediated gene repression and activation have been described in previous reviews (2,3). To repress the expression of a gene, the association of YY1 with a target promoter may cause activator displacement, interference with the activator's function or recruitment of corepressors. When activating a target gene, YY1 may directly act as an activator, inhibit the activity of repressors or recruit coactivators.

As a member of the GLI-Krüppel class of zinc finger proteins, YY1 has been reported to regulate the expression of a large cohort of genes and/or promoters. The four zinc fingers at the C-terminal of YY1 (Figure 1) are responsible for binding to its target promoters. The YY1 binding motif was initially recognized as having two types of cores,
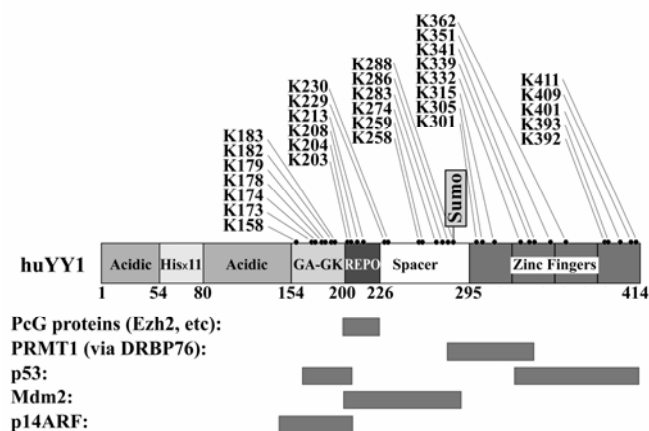

Figure 1. Domain structure of YY1 and interaction regions of histone methyltransferases and p53-related proteins. The YY1 sequence is based on the NCBI access number Z14077. All 32 lysine (K) residues are labeled on the top and the SUMO conjugation site, K288, is indicated. The REPO motif of YY1 is necessary and sufficient in recruiting PcG proteins, such as Ezh2, to YY1-targeted promoters to promote histone H3-K27 methylation and establish gene silencing (68). The residues 261-333 of YY1 are responsible for recruiting PRMT1 via DRBP76 to methylate histone H3-R4 (65). The interactions domains of p53, Mdm2 and p14ARF were mapped by *in vitro* protein binding experiments (47). For the binding domains of other YY1-interacting proteins, please refer to Seto's review (3).

ACAT and CCAT (29). Recently, longer DNA binding motifs were also identified (30). In an earlier report on the prevalence of YY1 binding sites, over 7% of vertebrate genes and 24% of viral genes contained YY1 binding elements (31), suggesting a ubiquitous regulation of YY1 in different biological processes. Interestingly, although YY1 was first recognized as a transcription factor, one report indicated that the localization of YY1 is cell cycle-dependent (32). In this report, the authors presented that YY1 mainly stayed in cytoplasm at G1 phase, but translocated into the nucleus at the early and middle S phases and then moved back to the cytoplasm at late S phase. The entry of YY1 into the nucleus coincided with increased YY1-DNA association and DNA/histone synthesis, suggesting a regulatory role of YY1 in cell division and proliferation. The function of YY1-regulated genes covers cell growth, proliferation, cytokinesis, apoptosis, development and differentiation, indicating that YY1 plays an essential role in coordinating multiple biological pathways through a complex transcriptional network (28).

In this review, I have categorized these targets into two groups based on the regulatory consequence of YY1: activation and repression. Because this review focuses on the genes involved in tumorigenesis, each group has also been divided into three subgroups based on the functions of the targeted genes or promoters (Tables 1.1 and 1.2).

2.1. YY1-activated gene expression

Many YY1-activated genes exert oncogenic or proliferative effects on cells (Table 1.1). The regulation of YY1 on some of these genes is reminiscent of its originally observed "Yin Yang" effects on the P5 promoter of adenovirus (7). c-Myc is the first oncogene that was demonstrated to be activated by YY1 (33). In this study, YY1 was shown to increase the levels of two major c-Myc mRNA transcript variants. A recent report further delineated the mechanism underlying this regulation and revealed that the viral oncogenic protein E1A plays a role in YY1-mediated activation of c-Myc expression. E1A disassociates the YY1-p300-HDAC3 complex, which initially inhibits c-Myc expression, and makes the c-Myc promoter more accessible by increasing regional histone acetylation (34). A similar scenario also occurs in the regulation of YY1 on another proto-oncogene, c-fos. Although YY1 can block c-fos gene expression through a direct interaction with the transcription complex ATF/CREB (35), this inhibition is also inverted by

E1A, which disrupts the ATF-CREB-YY1 complex and changes YY1 from a repressor to an activator of c-fos gene (36). Consistently, YY1 stimulates the association of serum response factor (SRF) with the c-fos serum response element (37), suggesting an activating function to c-fos gene expression. These studies indicate that YY1 does not activate, or may even repress, these proto-oncogenes in normal conditions. However, when stimulated by oncogenic signals, YY1 will facilitate tumorigenic processes.

Among the targets of YY1, several other genes also code oncogenic proteins. YY1 is highly expressed in breast cancer and stimulates the expression of ERBB2 (38,39). ERBB2 (also known as Her2 and neu) is overexpressed in about 30% of breast cancers and generally correlated with a poor prognosis (40). Since overexpressed ERBB2 contributes to increased aggressiveness of cancer cells, it is a notable target of breast cancer therapy. Increased ERBB2 partly results from its gene amplification (41), which might put the physiological significance of its upregulation by YY1 in doubt. However, a recent study showed that YY1 protein levels are inversely correlated with ERBB2 gene amplification in breast tumors (39), suggesting that YY1 may play a role in ERBB2 upregulation when this gene is not amplified.

As a prosurvival endoplasmic reticulum (ER) chaperone, GRP78/BiP has been shown in multiple studies to possess oncogenic properties by stimulating tumor proliferation, survival, metastasis, and resistance to various therapies (42). Baumeister et al demonstrated that YY1 associates with the GRP78 promoter only in conditions of ER stress. YY1 acts as an essential coactivator of ATF6 and recruits histone H4 methyltransferase PRMT1 (Figure 1) to enhance GRP78 gene expression (43). This study revealed an important role of YY1 in regulating stress-induced modification of chromatin and promoting cell survival response to stress conditions.

Other oncogenic or proliferative genes activated by YY1 include COX-2 and OTX2 (Table 1.1). It is noteworthy that two studies suggested the contribution of YY1 to the epithelial-mesenchymal transition (EMT). EMT can be induced by multiple oncogenic pathways and is inhibited by the tumor invasion suppressor E-cadherin. A recent report demonstrates the regulation of YY1 to the transcription of Snail, a transcriptional repressor of E-cadherin (44). The association between YY1 and the 3' enhancer of Snail is essential to Snail expression in melanoma cells, since decreased levels

Table 1.1. YY1-activated genes/promoters linked to tumorigenesis.

Gene/promoter	Function of the gene product	References
A. Oncogenic, proliferative and/or overexpressed genes in cancer		
c-Myc	Transcription factor and oncogene of various cancers	[33,139]
ERBB2/Her2	Proto-oncogene in breast cancer	[33,39]
COX-2	Oncogene of various cancers	[112]
GRP78/BiP	Promoting tumor proliferation, survival, metastasis and resistance to cancer therapies	[42,43,140,141]
OTX2	Oncogene of medulloblastoma	[142,143]
Snail	Enhancing cell survival, movement and/or EMT	[44,144]
Msx2	EMT and tumorigenesis	[45,145]
Mitochondrial genes: Cyto C etc	Cell respiration	[46]
DR-α	Overexpressed in cancers	[146,147]
VASAP-60/ PRKCSH/80K-H	Elevated in breast cancer	[148]
B. Tumor suppression genes		
ERGIC-53	Transmembrane lectin facilitating the efficient export of a subset of secretory glycoproteins from the endoplasmic reticulum; induced by ER-stress	[99]
HLJ1	Tumor and invasion suppressor	[53]
p53	Tumor suppressor	[51]
p73	A member of p53 family proteins	[52]
Peg3 (via CSE2 binding element)	Imprinted gene with tumor suppression function	[149]
RIZ1	A histone methyltransferase Altered expression in cancers, maybe a tumor suppressor	[150]
C. Other regulatory proteins in tumorigenesis		
Epidermal growth factor receptor (EGFR)	Cell signaling molecules involved in diverse cellular functions, including cell proliferation, differentiation, motility, and survival, and in tissue development	[151]
Histone H3.2alpha	Aberrant modifications in cancers	[77]
Histone H4	Aberrant modifications in cancers	[78]
Line-1 (promoter)	Showing altered methylation in cancers	[152]
Myelin Proteolipid protein (PLP)	Primary constituent of myelin in the central nervous system	[153]
OTK18	Induced by HIV infection	[154]
PARP-1	Promoting poly(ADP-ribosyl)ation; related to DNA damage repair	[76]
PCNA	Involved in DNA synthesis and repair; cooperating with nucleophosmin/B23	[80,155]
RE-1 silencing transcription factor (REST) *or* neuron-restrictive silencer factor (NRSF)	Showing both tumor suppressor and oncogenic activities	[156,157]
gp91(phox)	Catalytic subunit of the NADPH oxidase; potential target of cancer therapy	[158,159]
B-type natriuretic peptide (BNP)	Related to patients' response to cancer therapy	[160]
Transferrin receptor (CD71)	Related to poor prognosis and resistant to tamoxifen in breast cancer	[161]

Table 1.2. YY1- repressed genes/promoters linked to tumorigenesis.

Gene/promoter	Function of the gene product	References
A. Oncogenic and/or overexpressed genes		
c-fos	Proto-oncogene	[162]
interferon β (IFN-β)	Potential target in cancer therapy	[87,163]
HOXB13	Promoting cancer progression	[63,164]
CREB	Transcription factor	
matrix metalloproteinase-9 (MMP-9)	Increasing expressed in various cancers	[69]
steroidogenic acute regulatory (StAR)	Related to some cancers, e.g. glial tumors	[165,166]
B. Tumor suppression genes		
microRNA-29	Tumor suppressor of rhabdomyosarcoma	[57]
p21	Leading to cell cycle arrest	[55]
p16(INK4a)	Tumor suppressor	[56]
Peg3 and Usp29 (via CSE1 binding element)	Peg3: tumor suppression; Usp29: ubiquitin-specific protease 29	[149]
Rb	Tumor suppressor	[54]
TGF-beta	Tumor suppressor	
C. Other regulatory proteins in tumorigenesis		
alpha3beta1-integrin	Contradictory role in cancer invasion	[117]
mu opioid receptor (MOR)	Cancer-related pain	[102]
CD30	A member of the TNF receptor family; related to lymphoma	[167]
CXCR4	Chemokine receptor; related to breast cancer cell migration	[168]
PPAR-delta	Nuclear receptor proteins regulating gene expression	[169]
ERCC5/XPG	DNA repair gene	[170]
OX40	A therapeutic target in the treatment of autoimmunity and cancer	[171,172]
Cdk4	Regulating Rb phosphorylation	[55]
Cyclin D1	Regulating Cdk4 function	[55, 173]
Involucrin	A marker of differentiation	[174]
Hoxd4	Regulating morphogenesis	[175]

of YY1 led to reduced Snail expression. Another group also reported that YY1 acts as a transcriptional activator of a homeobox gene, Msx2, which plays an important role in inducing EMT transition in different cell types (45).

In 2007, a comprehensive study by Cunningham and colleagues described the role of YY1 in mediating cell respiration through mitochondria (46). YY1-binding elements are highly enriched in mitochondrial genes, and siRNA-mediated YY1 depletion significantly reduces the expression of many mitochondrial genes and in turn decreases the oxygen consumption. Consistently, YY1 protein is required for the inhibition of the mitochondrial genes by rapamycin. Mechanistic studies suggested that the YY1-PGC-1α transcriptional complex is essential to the mitochondrial oxidative function, while mTOR interferes with this regulation through altering the YY1-PGC-1α complex. Overall, this study demonstrated that YY1 plays an essential role in maintaining the basal respiration of cells.

Although the research described above implicates a proliferative role of YY1, several studies also indicated that YY1 may promote genes with tumor suppression function. While most reports demonstrated that YY1 negatively regulates p53 at posttranslational level and inhibits p53 transcriptional activity (47-50), YY1

exhibited stimulating effects in a study using a p53-promoter reporter and overexpressed YY1, and this activation was further enhanced by cotransfected E1A (51), which seems to contradict the well-established oncogenic function of E1A protein. Another report also demonstrated the cooperative transcriptional activation of p73 by YY1 and E2F1 (52). Overexpressed YY1, together with activator protein 1 (AP-1), was shown to activate the transcription of HLJ1, a suppressor of tumor invasion (53).

2.2. YY1-repressed gene expression

YY1 represses several targets with potential tumor suppression function. YY1 exhibits an inhibitory effect on Rb expression. During myogenesis (muscle cell differentiation), the translocation of YY1 from the nucleus to the cytoplasm causes Rb gene activation, which leads to the exit of cell cycle and the consequent myogenesis (54). However, it is still unclear whether overexpressed YY1 during tumorigenesis can inactivate the Rb gene. One way in which YY1 antagonizes p53 function is through attenuating p53 target genes, including p21 (49). The same regulation was also observed in vascular smooth muscle cells (55). YY1 was reported to recruit HDAC3 and HDAC4 to the promoter of p16(INK4a) and repress its expression, which could release the cells from senescence (56). YY1, together with NF-kappaB, could inhibit microRNA-29 (miR-29) (57), which is a potential tumor suppressor through activating p53.

Other YY1-regulated genes involved in tumorigenesis are shown in Tables 1.1 and 1.2. Based on the regulation of YY1 and the function of these genes, some discrepancies still exist, which makes it impossible to exclusively define the role of YY1 in cancer development. However, the tumorigenesis promoting effects of YY1 clearly override its function of tumor suppression. It is reasonable to hypothesize that the overall outcome of YY1-regulated processes depends on the oncogenic stimuli, cell types and the interplay with its recruited cofactors, whose availability may be altered at different physiological conditions.

3. YY1 and protein post-translational modifications

Posttranslational modifications have largely increased the complicity of the regulation on protein functions. Among several modifiable residues, lysines are the major targets of post-translational modifications, including acetylation, methylation, ubiquitination and sumoylation. The high-lysine (8%) composition of YY1 has determined its liability as a target of multiple modifying groups. Interestingly, these lysine residues are mostly located in the middle region of the primary sequence of the protein, while none is among the first 157 residues (Figure 1). While YY1 itself is a target, it also regulates various protein modifications. A well established mechanism of YY1-regulated gene expression is through recruiting histone modifiers to the target promoters and modulating histone modifications. YY1 has been reported to interact with numerous proteins including many protein modifiers that promote acetylation, deacetylation, methylation, ubiquitination and sumoylation of histone or nonhistone proteins (Table 2).

3.1. Acetylation

The interaction of p300 and YY1 was initially demonstrated by Lee et al in 1995 (58). Interaction domain mapping experiments revealed that YY1 and E1A bind to distinct sites of p300, and the primary binding sites of p300 and E1A on YY1 do not overlap, suggesting that the three proteins potentially form a ternary complex. Hence, the molecular mechanism underlying the conversion of YY1 from a repressor to an activator by E1A is through recruiting p300 to acetylate histones on YY1 target promoters. One of the transcription-independent properties of YY1 is its inhibition of p300-mediated p53 acetylation (48). This is one of the multiple approaches employed by YY1 to antagonize p53 (Figure 2), since acetylation can both prevent p53 ubiquitination (59) and enhance p53 transcriptional activity by promoting p53-DNA association (60).

Another landmark study by Yang et al linked the regulation of YY1 to histone deacetylation (61). They discovered an YY1 binding protein, hRPD3, a homolog of yeast RPD3 that is the definition base of the Class I HDACs. This hRPD3, which was later determined to be HDAC2 (3), contributes to YY1-repressed gene expression. Many other reports confirmed the importance of HDACs in YY1-mediated gene repression. Luke et al revealed that YY1 interacts with a homeodomain protein, HoxA11, and recruits HDAC2 to HoxA11 target gene to abrogate HoxA11-mediated gene activation (62). Recently, YY1 was reported to recruit HDAC4 and repress the expression of HOXB13, which plays a role in growth arrest in androgen receptor-negative prostate cancer cells (63).

Table 2. YY1 interacting proteins

Protein	Function	References
A. Modifiers of histones and nonhistone proteins		
p300, CBP	Acetylation of histone and nonhistone proteins	[58]
HDAC1, 2, 3, 4, 5	Deacetylation of histone and nonhistone proteins	[61,63,64,176,177]
Ezh1	Histone methyltransferease	[66]
Ezh2	Histone methyltransferease on H3-K27 and H1-K26	[67,178]
PRMT1	Histone methyltransferease on H4-R3	[65]
Mdm2	Ubiquitination of p53 and histones	[47,48]
Ubc9	E2 conjugating enzyme of sumoylation	[72]
PIASy	E3 ligase of sumoylation	[72]
B. Other chromatin remodeling proteins		
Nucleophosmin (NPM)/B23	A histone chaperon involved in nucleosome formation	[79]
CtBP1	A corepressor involved in chromatin remodeling	[81,82]
RYBP	A repressor present in PcG complex	[83]
CTCF	Chromatin remodeling; its deregulation causes epigenetic imbalance in cancer	[23,179]
INO80	Chromatin remodeling and DNA repair	[88,89]
SAP30	Involved in LOH (Loss of Heterozygosity)	[86,87]
C. Tumorigenesis and apoptosis		
p53	Tumor suppression and genome stability	[47]
Rb	Tumor suppression and genome stability	[91]
p14ARF	Tumor suppression	[47]
E1A	Oncogene leading to tumorigenesis	[7]
Mdm2	Oncogene enhancing p53 degradation	[47,180]
c-Myc	Oncogene transforming cells	[181]
c-Jun	Protooncogene	[94]
Caspases* 1, 3, 5, 6, 7	Proteases activated during apoptosis	[95]
PARP-1	Posttranslational modification, DNA repair	[76]
E2F2, E2F3	Regulating Rb pathway	[84]
MBP1	c-Myc promoter binding protein 1 (MBP-1)	[139]
α-enolase	Tumor antigen in lung cancer	[139]
D. General and other regulation		
RNA Pol. II, TFIIB, Sp1, ATF/CREB, TAFII55	General gene transcription	[35,96-99]
Notch1 receptor	Cell fate determination during embryonic development	[139,182]
SFMBT2	A member of PcG proteins	[183]
YY1AP (HCCA2)	Hepatocellular carcinoma-specific protein	[184-186]
YAF2	Interacting with MycN in neuroblastoma	[187,188]
mTOR (FRAP1)	Key factor in transducing various stimuli to regulate a wide range of cellular functions	[46]
Raptor	Associate with mTOR, and regulate its expression and activity	[46]
PGC-1α	A co-activator of YY1 in regulating mitochondrial genes	[46]
SHDAg and LHDAg (σ-virus antigen)	Involved in TGF-beta and c-Jun-induced signaling cascade	[189]
Hoxa11	Regulating uterine development	[62]
AP-2 (activator protein 2)	Acting as a co-factor to stimulate ERBB2 promoter	[38]
CP2	Transcription factor; interacting with the HXPR motif of YY1	[190]
SMAD1/4	TGF-beta signal pathway	[191]

The transcriptional activity of YY1 is also regulated by acetylation (64). p300 and PCAF mediate the acetylation of the central region (residues 171-200) of YY1 and this modification augments YY1-mediated gene repression. The C-terminal of YY1 can also be acetylated by PCAF, which reduces the YY1-DNA association.

3.2. Methylation

As stated above, YY1 is an activator of c-Myc gene. Rezai-Zadeh et al demonstrated that the mechanism of this activation is through the YY1-recruited PRMT1, a histone methyl-transferase specific to the arginine 3 of histone H4 (H4-R3) (65). The recruitment of PRMT1 by YY1 on the promoter of GRP78, a prosurvival ER chaperone, also leads to histone H4-R3 methylation and GRP78 gene activation (43). YY1 interacts with two lysine-specific histone methyl transferases, Ezh1 (enhancer of zeste homologue 1) and Ezh2, both of which mediate the methylation of histone H3-K27, a hallmark of gene silencing (66,67). Caretti et al first demonstrated that YY1 is essential to Ezh2-mediated methylation on histone H3-K27 in mouse skeletal muscle cells (67). This finding supports a regulatory role of YY1 in prostate cancer development, since the essential role of Ezh2 in prostate cancer development has been well established. Both YY1 and Ezh2 are overexpressed in various cancers. Thus, the recruitment of Ezh2 by YY1 may contribute to the aberrant epigenetic status of cancers. Transgenic studies in Drosophila indicated that the REPO (REcruitment of POlycomb proteins) motif, consisting of residues 201-226 of YY1 (Figure 1), is necessary and sufficient in recruiting Ezh2 and other polycomb group (PcG) proteins to establish transcriptional repression (68).

3.3. Ubiquitination and sumoylation

The turnover of YY1 is likely through ubiquitination and proteasomal degradation, since the treatment of a proteasome inhibitor led to accumulated YY1 protein (47). In neuronal cells, ubiquitinated YY1 interacts with CtBP and HDAC3 to establish a repressive complex (69). Meanwhile, we and others discovered that YY1 plays a role in regulating p53 ubiquitination and degradation (47,48) (Figure 2). YY1 directly interacts with both p53 and Mdm2, a ubiquitin E3 ligase, and enhances the Mdm2-p53 interaction by forming a ternary complex with them. As a result, YY1 depletion leads to either apoptosis or cell cycle arrest, depending on the cell types (28,47). Importantly, this regulation is
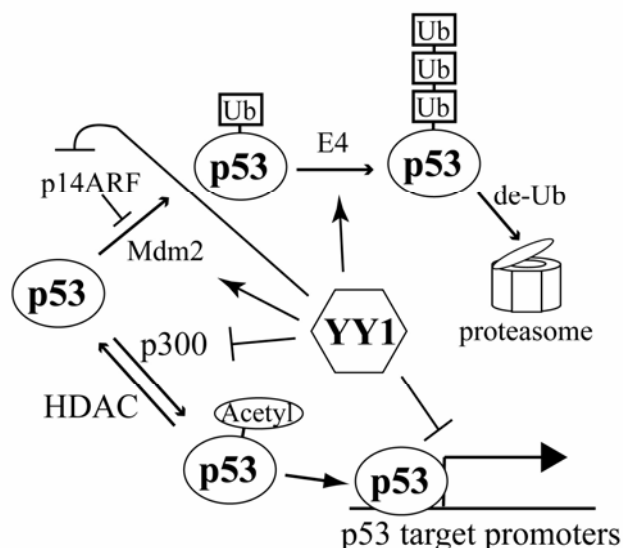

Figure 2. Schematic model of multiple mechanisms of YY1 to antagonize p53. YY1 inhibits p300-mediated p53 acetylation that endows p53 with increased stability and DNA binding affinity compared to deacetylated p53 [48]. YY1 also enhances Mdm2-mediated p53 ubiquitination and polyubiquitination, and interferes with p14ARF-mediated p53 stabilization [47]. In addition, YY1 also inhibits the transcription of p53 target genes, such as p21. Acetyl: acetylation; Ub: ubiquitination; de-Ub: deubiquitination driven by ubiquitin-specific proteases.

independent to the transcriptional activity of YY1, since an YY1 mutant deficient in DNA binding retains the ability of stimulating p53 ubiquitination, and YY1 protein purified from a bacterial expression system is capable of enhancing p53 ubiquitination *in vitro* (47).

The functional outcomes of protein sumoylation and ubiquitination are very distinct (70). SUMO (Small Ubiquitin-related MOdifier) conjugations are normally present in specific regions responsible for protein-protein interactions. Therefore, sumoylation may alter the function of a protein by changing its binding partners (71). We have demonstrated that YY1 can be conjugated by SUMO-1, 2 and 3, and Lys288 of YY1 is the primary conjugating site (Figure 1) (72). YY1 sumoylation, which is stimulated by PIASy, a SUMO E3 ligase, negatively affects the transcriptional activity of YY1. In addition, we also revealed a direct interaction of YY1 with Ubc9, the only conjugating enzyme of protein sumoylation. The interaction of YY1 with Ubc9 and PIASy suggests a potential role of YY1 in regulating the sumoylation of its interacting partners.

The interaction of YY1 with different transcription factors and protein modifiers also contributes to YY1-mediated gene expression. As

schematically shown in Figure 3, multiple studies demonstrated the regulation of YY1 to histone acetylation, deacetylation and methylation by p300, HDACs, Ezh2, Ezh1 and PRMT1, respectively. It is possible that these modifications occur on non-histone proteins in an YY1-recruited transcriptional complex. In addition, Mdm2 and Ubc9 may also be recruited by YY1 to its targeted promoters and modify these cofactors to regulate gene expression.

3.4. Other modifications

YY1 is also a subject of other modifications. Some of the YY1 present in nucleus appears to be O-GlcNAcylated regardless of the differentiation status of the cells, and glycosylated YY1 no longer interacts with Rb, although it still binds DNA (73). This study suggested that the glucose metabolism regulates YY1 protein by promoting its O-GlcNAcylation and consequently changing its activity. Although glycosylation frequently occurs to proteins expressed in cell membrane, it also plays an important role in regulating transcription. A number of transcription factors, such as Sp1 and RNA polymerase II, bear glycosylation that affects their transcriptional activity (74). Whether the glycosylation affects YY1-mediated transcription, in addition to its interaction with Rb, is still unclear. Moreover, YY1 interacts with poly (ADP-ribose) polymerase 1 (PARP1) and stimulates its function in catalyzing the synthesis of ADP-ribose polymers (75). Meanwhile, YY1 was reported to be transiently poly(ADP-ribosyl)ated after genotoxic treatment, which is coincides with the activation of PARP1 (76). Unlike glycosylation, the poly(ADP-ribosyl)ation decreased the DNA-binding affinity of YY1.

4. YY1-interacted proteins and their function

YY1 has been reported to interact with numerous proteins and most of these interactions are direct. One report suggested that YY1 may change its subcellular localization at different stages of the cell cycle (32). We observed that YY1 is predominantly localized in the nucleus but a small portion of YY1 also can be found in the cytoplasm, suggesting that its binding proteins are present in both compartments and YY1 possibly switches its binding partners during cell division and proliferation. The proteins that interact with YY1 can be categorized into four groups (Table 2), most of which possess regulatory function in tumorigenesis.

4.1. Protein modifiers

This has been discussed in Section 3 and summarized in Table 2.
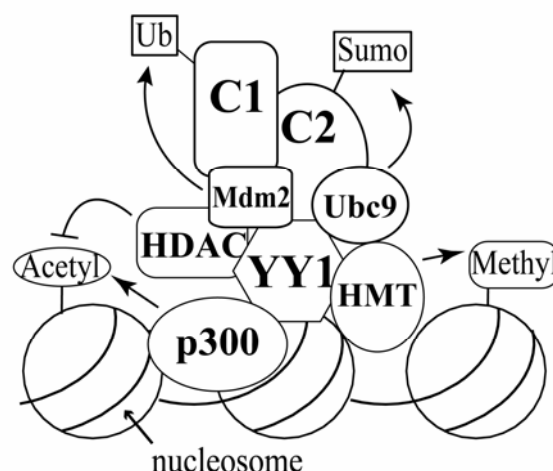

Figure 3. Schematic model of YY1-mediated protein modifications of histone and non-histone proteins on its target promoter. The interaction of YY1 with protein modifiers may mediate the modifications of the components in an YY1-recruited transcriptional complex. YY1 recruits p300, HDAC and histone methyltransferase (HMT, such as Ezh2, Ezh1 and PRMT1) to acetylate, deacetylate and methylate histones, respectively. Function of other cofactors (designated as C1 and C2) may also be altered by YY1-mediated modifications, such as ubiquitination and sumoylation.

4.2. Other chromatin remodeling proteins

In addition to these protein modifiers that directly regulate histone modifications, a number of other YY1-interacting proteins are also involved in chromatin remodeling. YY1 directly regulates the gene expression of histone H3.2alpha and histone H4 (77,78). These histone proteins are the primary components of the nucleosome. As a nucleolar protein and histone chaperone, nucleophosmin (NPM)/B23 regulates nucleosome formation. NPM forms a complex with YY1 both *in vivo* and *in vitro*. Interestingly, NPM relieves the transcriptional repression mediated by YY1 (79) and promotes the YY1-mediated activation of PCNA promoter (80). Among the polycomb group (PcG) proteins, YY1 is the only member that is known to directly and specifically bind DNA and recruit other PcG proteins to establish gene silencing. Using Drosophila as a model, the Atchison group demonstrated that the gene repression mediated by YY1 and other PcG proteins requires the co-repressor CtBP (81,82). A recent study in rat hippocampal cells indicated that CtBP specifically interacts with mono-ubiquitinated YY1 to repress matrix metalloproteinase-9 (MMP-9) gene transcription (69). Therefore, YY1 deubiquitination resulted from neuronal depolarization disrupts the repressive complex of

YY1-CtBP-HDAC3 and in turn activates MMP-9 expression.

RYBP (Ring1- and YY1-binding protein) was initially identified as a corepressor in PcG complex (83). A later study indicated that RYBP mediates the interaction of YY1 with E2F protein, which leads to activation of the Cdc6 gene (84). At the protein level, RYBP antagonizes Mdm2-mediated p53 ubiquitination (85), in opposition to the effects of YY1.

The interaction between YY1 and SAP30, a component of the human histone deacetylase complex, has been proposed as an alternative mechanism of YY1-mediated gene repression (86). SAP30 promotes the recruitment of HDAC1 by YY1 to repress gene expression. In addition, the recruitment of the Sin3A/NCoR/HDACs repressor complex by YY1 inhibits the expression of interferon beta (IFN-beta) gene (87).

INO80 is a subfamily of SWI2/SNF2 chromatin remodeling proteins and plays regulatory roles in gene transcription, DNA repair and DNA replication. Two recent studies demonstrated the functional interplay between YY1 and INO80. When YY1 activates transcription of its target genes, INO80 acts as an essential co-activator and helps YY1 to gain access to the target promoters (88). In addition, YY1 and INO80 are essential to homologous recombination-based DNA repair and therefore may regulate the cellular response to genotoxic stress (89).

4.3. Proteins involved in tumor suppression, oncogenesis, apoptosis and DNA damage

Besides p53 and Mdm2, YY1 also interacts with many other proteins that directly regulate tumorigenesis. YY1 binds to tumor suppressor retinoblastoma (Rb) *in vitro*. Either glycosylation of YY1 or phosphorylation of Rb disrupts this interaction (73,90). In cell-based experiments, only hypophosphorylated Rb interacts with YY1 and this interaction disrupts the YY1-DNA association (90), suggesting that Rb may be involved in YY1-mediated transcriptional regulation. Interestingly, the YY1-Rb complex was only observed in resting cells, but not in serum or lipopolysaccharide (LPS) stimulated cells (90,91), indicating their interaction is cell cycle-regulated. Another YY1-interacting tumor suppressor is p14ARF. Our functional study demonstrated that YY1 competes with p14ARF and therefore attenuates p14ARF-mediated p53 activation (47), which is another way in which YY1 antagonizes p53 function (Figure 2).

YY1 also interacts with many oncogene products, including E1A, c-Myc, c-Jun and Mdm2. As described above, viral oncogene E1A converts YY1 from a transcriptional repressor to an activator. As an YY1 interacting protein, c-Myc prevents YY1 from associating with its cofactors, but does not block its binding to DNA (92). YY1-c-Myc interaction mediates the stimulation of Surf-1 in the MAP kinase cascade (93). Similarly, c-Jun also interacts with YY1 and decreases the binding affinity of YY1 to its consensus binding element (94).

Krippner-Heidenreich et al provided direct evidence of the involvement of YY1 in apoptosis (95). Various apoptotic stimuli could promote rapid translocation of YY1 into cell nucleus and lead to cleavage of YY1 at Asp12-Gly and Asp119-Gly. Interestingly, one of these N-terminal truncated forms of YY1 could enhance Fas-induced apoptosis, suggesting YY1 plays a role in positive feedback during apoptosis. An *in vitro* study showed that YY1 was cleaved by caspase 1, 3, 5, 6 and 7 (95).

4.4. Other regulatory proteins

YY1 interacts with many general transcriptional factors and cofactors, such as RNA polymerase II, ATF/CREB, and Sp1 (35,96-99). This suggests that YY1 not only recruits other transcriptional cofactors to its target promoters, but also potentially acts as a cofactor recruited by others. Importantly, the presence of YY1 in a transcriptional complex creates an interface for these YY1-interacted protein modifiers that may alter the function of transcriptional machinery by modulating their posttranslational modifications (Figure 3). The interactions of YY1 with these general transcriptional factors and the regulatory proteins in Notch, TGF-beta, mTOR signaling pathways, as well as cell respiration (Table 2), once again indicate a critical role of YY1 in different biological processes.

5. YY1 being regulated

Although most reports have described how YY1 regulates the expression or modifications of other genes or proteins, some studies also demonstrated how YY1 expression and function are regulated (Table 3).

YY1 expression was shown to be upregulated by two growth factors, insulin-like growth factor-1 (IGF-1) and fibroblast growth factor-2 (FGF-2) (100,101). YY1 exhibited low or absent expression in NIH3T3 cells that were quiescent or cultured in serum-deprived medium. Consistently, YY1 expression could be equally stimulated by serum or

Table 3. Factors that regulate the function of YY1

Factor	Effect on YY1	References
Insulin-like growth factor-1 (IGF-1)	Activation	[100]
Fibroblast growth factor-2 (FGF-2)	Activation	[101]
TNF-alpha/NF-kappaB	Activation	[104,105]
Morphine	Stimulating YY1 expression	[102]
Lysophosphatidylcholine (lysoPC)	Enhancing YY1 expression	[103]
C/EBP-beta	Inducing YY1 activity	[192]
Prohibitin	Repressing YY1 expression	[107]
MicroRNA-29	Inhibiting YY1 translation	[57]
DETANONOate (nitric oxide donor)	Inhibiting YY1 mRNA synthesis and YY1-DNA association	[109,110]
Staphylococcal enterotoxin A (SEA)	Reducing YY1 expression by 15-fold in peripheral blood	[111]
Naloxone	Downregulating YY1 expression	[102]
RKIP	Down-regulating YY1 via NF-kappaB inhibition	[108]
Rituximab	Inhibiting YY1 expression	[106]
Lipopolysaccharide (LPS)	Inducing YY1 binding to COX-2 promoter	[112]
Sumoylation/PIASy	Enhancing YY1 sumoylation	[72]
Ubiquitination	Enhancing DNA binding and interaction with CtBP, HDAC3	[69]
Different apoptotic stimuli and DNA synthesis inhibitor	Causing YY1 translocation and cleavage	[32,95]
Myeloid nuclear differentiation antigen (MNDA)	Increasing YY1 affinity to DNA	[113]

IGF-1 (100). Injury of smooth muscle cells also led to an increase of YY1 expression; this stimulation was abolished by treatment with FGF-2 antibody (101). Proliferative drugs, such as morphine and lysophosphatidylcholine, also increased YY1 expression (102,103).

YY1 expression is stimulated by transcription factor NF-kappaB that directly binds to YY1 promoter through its subunit p50/p65 heterodimer (104). Consistently, PC-3 cells treated by TNF-alpha exhibited elevated levels and increased DNA-binding activity of YY1. On the other hand, genetic ablation of the p65 subunit of NF-kappaB in both cultured cells and adult skeletal muscle correlated with reduced YY1 transcripts and protein (105). A chimeric antibody against CD20, rituximab, which inhibits constitutive NF-kappaB activity and therefore sensitizes tumor cells in B cell non-Hodgkin's lymphoma and leukemias, represses YY1 expression (106).

Several mechanisms have been reported to downregulate YY1 expression. At the transcriptional level, YY1 expression is inhibited by prohibitin through E2F1 binding sites (107). While YY1 negatively regulates miR-29 expression, miR-29 also targets the 3'-UTR of YY1 mRNA and blocks its translation (57). The interplay between YY1 and miR-29 implicates their function in skeletal myogenesis and rhabdomyosarcoma development.

As a metastasis suppressor gene, Raf kinase inhibitor protein (RKIP) is poorly expressed in cancers. RKIP downregulates YY1 expression through inhibiting its transcription (108). Hence, RKIP overexpression increases tumor cell sensitivity to TRAIL via blocking YY1 expression. YY1 expression can also be inhibited by DETANONOate (a nitric oxide donor), naloxone (a drug used to counter the effects of opioid overdose) and staphylococcal enterotoxin (102,109-111).

In addition to regulating YY1 expression, some factors also affect YY1 function. Both lipopolysaccharide (LPS) and myeloid nuclear differentiation antigen (MNDA) enhance YY1 binding affinity to its target promoters (112,113). Mono-ubiquitinated YY1 recruits CtBP and HDAC3 to repress Mmp-9 gene expression (69), while PIASy-mediated sumoylation inhibits YY1-mediated gene transcription (72). Different apoptotic stimuli, including the DNA synthesis inhibitor aphidicolin, can translocate and cleave YY1 protein (32,95).

Overall, YY1 expression is stimulated by proliferative stimuli, while antiproliferative signals tend to antagonize YY1.

6. YY1 expression in cancers and its correlation with disease outcome

Most processes mediated by YY1 are cancer-related, while YY1 itself is also differentially regulated during apoptosis and tumorigenesis. Therefore, the studies summarized above provide unequivocal evidence for the essential role of YY1 in tumorigenesis. The regulatory role of YY1 in various signaling pathways may explain its aberrant expression in cancers. YY1 overexpression has been demonstrated in human breast cancer (38), prostate carcinoma (114), acute myeloid leukemia (115), osteosarcoma (116,117), cervical cancer (118), brain cancer (119), ovarian cancer (120), large B-cell and follicular lymphoma (121) and colon cancer (122). Currently, the mechanisms of YY1 increase in these cancers remain unclear. As discussed in Section 5, YY1 expression can be regulated by multiple pathways and its upregulation can be achieved by different proliferative stimuli. Therefore, it is unlikely that a universal mechanism can be applied to YY1 upregulation in all cancers.

In prostate cancer, elevated YY1 expression correlates with higher morphologic grades or malignant histological phenotypes (114). Similarly, YY1 overexpression in osteosarcoma is positively and strongly correlated with the degrees of malignancy (116,123). Consistently, YY1 is also upregulated in metastatic breast cancer (124) and its staining intensity in colon cancer is more pronounced in poorly differentiated tumors than in moderately or well-differentiated colon cancers (122).

As discussed above, the overall effect of YY1 on cells is proliferative or oncogenic, and YY1 overexpression is prevalent in most cancers. However, the expression of YY1 does not show a defined correlation with the susceptibility of cancer reoccurrence or length of patients' survival. In prostate, colon and ovarian cancers, YY1 expression positively correlated with the long-term survival periods of patients (114,120,122,125). In contrast, among patients with large B-cell and follicular lymphoma, high levels of YY1 were associated with poor outcome, including a shorter survival interval (121). The mechanisms of this apparent paradox remain unknown. A recent study by Matsumura et al investigated the positive correlation between YY1 expression and the length of survival in patients with ovarian cancer (125). Their results suggested that the overexpression of YY1 and E2F could sensitize ovarian cancer cells to the treatment of taxane, a class of drugs that disrupt the function of microtubules. As a result, patients with higher expression of YY1 exhibited better response to the therapy and therefore had longer survival.

Despite the fact that YY1 exhibits oncogenic function in cancer development, an *in vitro* study using colon cancer cells showed no evidence of gene amplification or chromosomal translocation of YY1 (122). However, two YY1 mRNA isoforms (7.5 and 2.9 kb) were substantially overexpressed and aneuploidy was also observed.

7. The role of YY1 in tumorigenesis and its potential as a target in cancer therapy

The essential regulation of YY1 in numerous cancer-related pathways and its increased expression in various cancers strongly implicate the importance of YY1 in cancer development and progression.

As a guardian of the genome, the tumor suppressor p53 plays a vital role in preventing malignant transformation of cells. Consistently, p53 mutations have been reported in over 50% of cancers. *In vitro* and *in vivo* studies also indicated that p53 inactivation can immortalize the cells and lead to deregulated cell proliferation or tumor formation. As described above, YY1 antagonizes p53 through several mechanisms (Figure 2), including enhancing p53 ubiquitination and degradation (47), blocking p53 acetylation (48), attenuating p14ARF-mediated p53 stabilization (47), and inhibiting p53-mediated transcription (49). These multiple and consistently negative effects of YY1 on p53 stability and function implicate p53 as a primary target of overexpressed YY1 in cancer cells and strongly suggest an oncogenic or proliferative role of YY1 in cancer development.

We hypothesize that YY1 coordinately regulates the function of Ezh2 and p53 to facilitate cancer development. Ezh2 has been identified as a bona fide oncogene (126) and used as a marker of cancers with aggressive and metastatic potential. Functionally, Ezh2 is essential to cancer progression and invasion (127,128), and its overexpression increases the likelihood of therapy failure (129). In prostate cancer, although p53 deficiency did not lead to prostate carcinoma in a mouse model, p53 deletion augments the cancer development in Pten null mice (130). Our proposed model is that the regulation of p53 and Ezh2 by YY1 may take place at different stages of cancer development (Figure 4).

Firstly, since nearly half of the cancers retain functional p53, especially at early stages, a developing phase must exist in the primary malignancy to defeat the genomic surveillance or tumor suppression function of p53 prior to its inactivation. We hypothesize that at this earlier stage, highly expressed YY1 plays a role to antagonize p53 function, which consequently initiates and/or promotes cell malignancy. Meanwhile, the suppression of Ezh2 expression by p53 (131) will also be released by YY1-mediated p53 inactivation and this in turn leads to increased levels of Ezh2 (Figure 4). Secondly, Ezh2 increase is always linked to tumor progression, invasion and metastasis (132-134), which are the later events of cancer development. We hypothesize that elevated YY1 expression is essential for Ezh2 to exert its methyltransferase activity and establish aberrant epigenetics, which augments cancer progression. At this stage, most of the cancers may already have acquired p53 mutations, which will augment the cancer progression. To these retaining functional p53, YY1 may still play a role in attenuating its function.

Noteworthily, our model suggests that YY1 antagonizes the functional p53 during the cancer development. This hypothesis does not conflict with the Vogelstein model for colorectal tumorigenesis (135,136), which proposed that the acquired p53 inactivation, mostly through gene mutations, is a frequent event at the late stages of cancers. Based on the published data, YY1 is likely an important negative regulator of p53 throughout the whole malignant process, especially in the cancers preserving functional p53 proteins. Certainly, we cannot exclude the possibility that the contribution of our model to tumorigenesis is more important in certain types of cancers than the others.

Several studies described the potential of YY1 as an effective target in cancer therapy. The Bonavida group extensively studied the role of YY1 in chemo- and immuno-resistance in cancer therapy and concluded that YY1 levels could be used to predict the therapeutic responsiveness (137). Since YY1 negatively regulates the expression of Fas, the inhibition of YY1 by nitric oxide or rituximab could upregulate Fas and sensitize the tumor cells to Fas-induced apoptosis (110,138). In addition, YY1 also inhibits the expression of DR5, a death receptor mediating the extrinsic pathway of apoptosis. Therefore, Raf-1 kinase inhibitor protein (RKIP) could decrease YY1 expression and consequently upregulate DR5 to sensitize the cells to TRAIL-
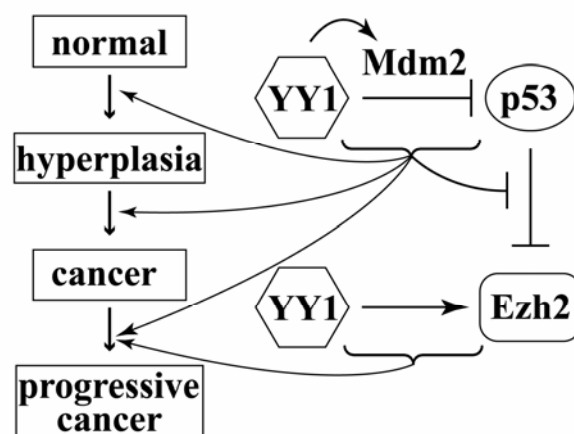

Figure 4. Schematic model of the regulation of p53 and Ezh2 by YY1 in cancer development. YY1 promotes Mdm2-mediated p53 degradation [47,48,55] and inhibits its transcriptional activity [49]. This leads to tumor initiation and cancer development. Moreover, the suppression of p53 on Ezh2 gene expression [131] will also be released by YY1's antagonism to p53, which results in increased Ezh2 expression. At the late stage of cancer, YY1 recruits Ezh2 for histone methylation and consequently promotes tumor progression. Meanwhile, overexpressed YY1 may also inhibit p53 activities in cancers retaining functional p53 proteins.

induced apoptosis (108).

A recent report by de Nigris et al focused on the role of YY1 in cell invasion, angiogenesis and metastasis (123). Their study demonstrated that YY1 depletion significantly decreased cell invasion and metastasis growth, which was associated with reduced endothelial growth factor (VEGF) and angiogenesis. This finding clearly suggests that YY1 is a promising and effective target in the therapy of bone cancer.

As summarized above, although discrepancies still exist, the overall function of YY1 is understood as both inhibiting tumor suppression processes and promoting oncogenic events. Especially, YY1 is an essential regulatory factor of numerous epigenetic events and its expression may affect many different biological processes leading to tumorigenesis. Theoretically, simultaneously targeting several pathways related to cancer development should result in more efficient and prompt outcome than targeting each of them individually. If a regulatory protein contributing to the abnormality of several processes toward malignancy can be identified, targeting this key regulator may exhibit a substantial impact by reversing or adjusting

multiple pathways concurrently. Given its unique properties in mediating multiple epigenetic events and its causal links with various cancers, YY1 is likely one of these key regulatory proteins in cancer development and therefore can serve as an effective target in therapeutic treatment of cancers. Thus, targeting or adjusting YY1 in cancer therapy can potentially reverse the aberrant epigenetics of cancer cells and restore their normality. This will be especially important to the cancers in critical organs where radical surgery is not applicable.

Acknowledgement

Conflicts of Interest

No potential conflicts of interest to disclose.

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Yin Yang 1: a multifaceted protein beyond a transcription factor

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Abstract: As a transcription factor, Yin Yang 1 (YY1) regulates the transcription of a dazzling list of genes and the number of its targets still mounts. Recent studies revealed that YY1 possesses functions independent of its DNA binding activity and its regulatory role in tumorigenesis has started to emerge.

Running title: YY1, not just a transcription factor

Keywords: Yin Yang 1, transcription factor, coactivator, protein modification, androgen receptor, prostate cancer.

YY1 was discovered as a transcription factor and its transcriptional activity can be converted from a repressor to an activator by viral oncoprotein E1A[1]. As a ubiquitously expressed transcription factor, YY1 has been demonstrated to regulate cell proliferation and differentiation, and its deficiency in mice results in peri-implantation lethality during embryonic development[2]. However, YY1 has attracted the interest of researchers not only because of its essential role in normal cell growth, but also its aberrant expression and potential regulatory function in different cancers. YY1 has been reported to modulate a mounting list of genes, many of which are key players in different signaling pathways regulating cancer development and progression, such as c-myc, c-fos, ERBB2, E1A and p53[3, 4]. Meanwhile, YY1 also physically interacts with a number of proteins regulating cell proliferation and apoptosis, such as p53, Mdm2, Ezh2, Rb, caspases and HDACs[3]. In addition, YY1 gene expression can be stimulated by several growth factors, while antiproliferative signals tend to antagonize its expression[3]. Therefore, as a multifunctional mediator of different signaling pathways, YY1 potentially acts as a critical regulator in cancer development and progression, and likely plays a proliferative or oncogenic role in these processes.

1. YY1: beyond a transcription factor

To date, most published data have demonstrated the activities of YY1 as a transcription factor. YY1 can either activate or repress gene expression, depending on the cofactors that it recruits. The promoters of these genes normally contain at least one of the two most frequent core binding elements of YY1, CCAT and ACAT[5]. YY1 plays an essential role in embryonic development[2]. Consistently, many YY1-targeted genes are key regulators of cell growth and differentiation, such as several mitochondrial proteins, Cdc6 and cyclin D1[3]. A major portion of these targets are key components of signaling pathways related to apoptosis and oncogenic transformation[3, 4].

Despite the well-established regulation of YY1 as a transcription factor, several reports, including ours, have demonstrated the role of YY1 as a transcription cofactor and its activity independent of its DNA binding. In our recent study, we observed an YY1-dependent expression of prostate-specific antigen (PSA) in prostate cancer cells[6]. Due to the well-recognized function of YY1 as a transcription factor, we first tested whether YY1 directly regulates PSA

transcription. When we mutated the only YY1 binding element in the PSA promoter, YY1-mediated transcription of PSA promoter was unaffected. Since androgen receptor (AR) is a well-studied transcription activator of PSA, we therefore asked whether YY1 is involved in AR-mediated transcription of PSA. Following this path, we discovered that YY1 directly interacts with AR and enhances the association of AR with an androgen response element (ARE). Importantly, we observed that an YY1 mutant deficient of binding to AR lost the stimulating effect on the PSA promoter that wild type YY1 has. Hence, these studies suggest that YY1-AR interaction, but not YY1-DNA association, is essential for YY1-activated PSA expression. Therefore, instead of directly acting as a transcription factor, YY1 plays a role of a cofactor and its DNA binding activity is dispensable in mediating PSA expression. It is noteworthy that AR interacts with the C-terminal of YY1[6], where its DNA binding domain, the zinc finger region, is located. Therefore, it is unlikely that YY1 molecule can associate with its DNA binding element and AR protein concurrently. The domain mapping studies indicate that the N-terminal of YY1 possesses transcriptional activation activity, and the N-terminal and middle region recruit many proteins with chromatin remodeling functions[7, 8]. Therefore, with the C-terminal binding to AR, the N-terminal of YY1 will likely recruit other coactivators and consequently promote PSA gene transcription.

Actually, this is not the first observation of YY1 exhibiting activity independent of its DNA binding affinity. Our previous study also indicated that YY1 enhances the Hoxa11-DNA association and is required for the recruitment of HDAC2 by Hoxa11[9]. Moreover, we and others reported that YY1 stimulates Mdm2-mediated p53 ubiquitination and degradation[10-12]. In this study, both wild type YY1 and its DNA-binding deficient mutant promoted p53 ubiquitination. Importantly, this effect of YY1 on p53 could be visualized in a reconstituted ubiquitination system *in vitro*[10]. Additionally, the observation of altered translocation between nuclear and cytoplasm at different cell cycle stages also suggests that YY1 is involved in biological processes in addition to mediating gene transcription[13-15].

The discovery of YY1 as a cofactor of AR extended our understanding of YY1-mediated transcription. YY1 interacts with a number of protein modifiers that mediate various posttranslational modifications. Early studies by Shi and Seto demonstrated the association of

YY1 with p300 and HDACs regulating histone acetylation and deacetylation, respectively[16, 17]. Studies by Sartorelli and Seto groups also revealed that YY1 recruits Ezh2 and PRMT1 to mediate histone methylation on lysine and arginine residues, respectively[18, 19]. In addition, our recent studies demonstrated the association of YY1 with Mdm2, PIASy and Ubc9 that regulate protein ubiquitination and sumoylation, respectively[10, 20]. Therefore, when binding to its own responsive element or recruited by another transcription factor, YY1 can likely provide a platform for the assembly of a scaffold with different transcriptional machineries where many other cofactors are recruited and assembled together. Especially, when a particular protein modifier binds to YY1, posttranslational modifications may occur to both histones and other recruited cofactors on a target promoter to modulate their functions. This interplay between the recruitment and modification will therefore determine the expression status of the regulated genes. Generally, p300-mediated histone acetylation and PRMT1-mediated histone H4-R3 methylation cause gene activation, while histone deacetylation by HDACs and H3-K27 tri-methylation by Ezh2 silence target genes (Figures 1A and 1B). In addition, Mdm2 can cause histone ubiquitination and establishes transcriptional repression[21]. Most transcription factors, such as AR[22-24], p53[25] and YY1 itself[3], also undergo various modifications, which differentially regulate their transcriptional activities. When YY1 acts as a transcription coactivator in AR-mediated PSA gene expression, it is likely that YY1 recruits other coactivators that are yet to be identified to promote transcription (Figure 1C). It is noteworthy that, as a cofactor, the cellular levels of YY1 may determine the expression status of a target gene. Therefore, while a medium increase of YY1 stimulates AR-mediated transcription, further increases of YY1 can compromise this activation[6]. One possible reason for this phenomenon is that excessive YY1 associates with other coactivators to be recruited and therefore interferes with the transcriptional activation of a target gene, the so-called "squelching effect"[26].

2. YY1: involvement in prostate cancer
It has been suggested that YY1 has a regulatory role in cancers based on the properties of its target genes and interacting proteins. Moreover, YY1 overexpression has been detected in multiple cancers, including prostate cancer[3]. Bonavida group made a number of seminal discoveries that link YY1 to prostate cancer development and therapy[4]. They demonstrated a significant association between YY1 activity and the expression of both cytokine and death

receptor. Mechanistically, YY1 negatively regulates the expression of both Fas and the death receptor 5 (DR5), and consequently endows prostate cancer cells with resistance to Fas-induced or TRAIL-induced apoptosis. Consistently, in a tissue microarray study, they observed that elevated YY1 expression correlates with higher morphologic grades or malignant histological phenotypes in prostate cancer samples from 246 patients[27]. Our finding of YY1 as a coactivator of AR in promoting PSA transcription also implicates that YY1 potentially regulates prostate cancer development and progression through stimulating AR function. Especially, in the absence of androgen, we observed a modest activation by YY1 on AR-dependent transcription of the PSA promoter[6], suggesting that, as a coactivator, overexpressed YY1 may be an essential regulator to the AR-signaling pathway in androgen independent prostate cancer.

Prostate cancer is a major public health problem among men of many countries. While locally confined prostate tumors can be treated by surgery and radiation therapy, advanced and relapsed prostate cancers are primarily handled by inhibiting the androgen signaling pathway. This can be typically achieved by androgen deprivation therapy including castration and administration of androgen antagonists. However, despite these therapies of androgen withdrawal, prostate cancer unavoidably progresses to the androgen-independent state. Even at low androgen levels, androgen-responsive genes in these tumors restore their expression nearly to the pre-treatment levels[28]. This strongly suggests that AR still plays an essential role in the growth and survival of recurred or advanced prostate cancers, even in androgen-deprived conditions. Actually, such prostate cancers retain high expression levels of AR and are still AR-dependent[29]. Therefore, AR is still a valid target for the therapeutic intervention of androgen-independent prostate cancers.

The aberrantly activated AR-signaling pathway in prostate cancer can be caused by different deregulations, including AR overexpression, AR mutation, increased sensitivity of AR to low androgen levels and ligand-independent AR activation[30]. Several mechanisms may lead to enhanced AR activity or sensitivity, including posttranslational modifications and stimulation of overexpressed coactivators[30]. Our observation on that overexpressed YY1 enhances the transcriptional activity of AR suggests that YY1 and its interaction with AR or other transcription cofactors can be potential, alternative targets in the therapeutic treatment of androgen independent prostate cancer. Given the fact that many coactivators and corepressors

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interact with YY1, specific agonists or antagonists to these proteins can be designed and tested, and molecular agents, such as peptide inhibitors, can be used to attenuate AR activity. These studies can potentially lead to the development of new therapeutic strategies that are urgently needed to treat the fatal, advanced prostate cancer.

Figure legend

Figure 1. Schematic models of YY1 regulated gene expression. YY1 can act as transcription activator (A), repressor (B) and coactivator (C, with the PSA promoter as an example). Other coactivators (designated as CoAc1 and CoAc2) can be recruited by YY1 to facilitate AR-mediated PSA transcription.

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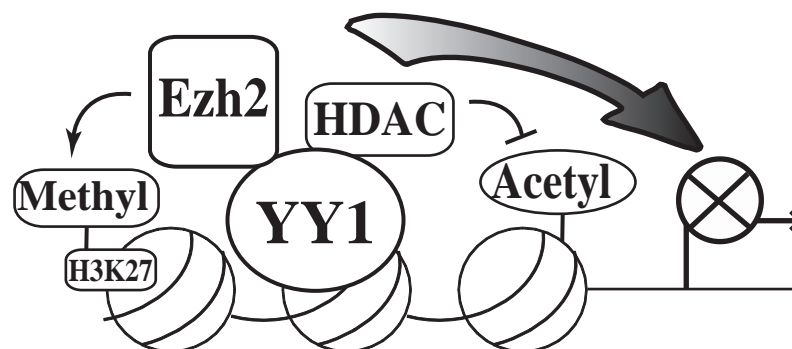
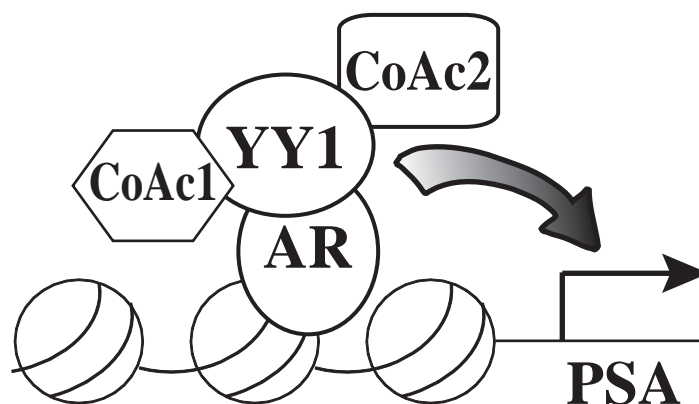**Figure 1.**   **Deng, et al**

A. Activation
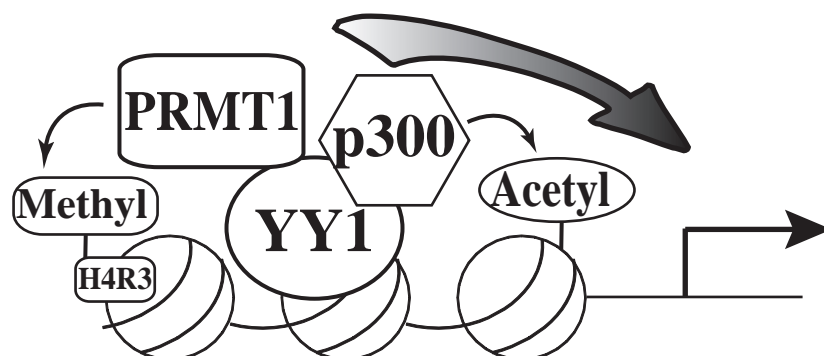
B. Repression

C. AR-YY1-mediated
   PSA trascription

Curriculum Vitae

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1. Generally, I am interested in how aberrant epigenetic regulation can mediate the initiation, development and progression of cancers. I am focusing on YY1-regulated pathways, including Mdm2-mediated p53 degradation and Ezh2-mediated histone methylation.

2. Specifically, I am investigating how YY1 expression can affect prostate cell proliferation *in vitro* and prostate cancer development/progression *in vivo*. I am also interested in breast cancer and microRNA studies and want to extend my research area to these fields.

3. I am interested in the functional study of microRNAs and their regulation in prostate cancer development. Especially, I want to identify the regulatory roles of microRNAs that are frequently deleted in prostate cancer.

PATENTS:
Pending: Yang Shi and **Guangchao Sui**. Compositions and Methods of Suppressing Eukaryotic Gene Expression. Patent Application 10/301,516, Harvard Case 2032.

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Title of Talk: Pursuing the epigenetic role of YY1 in cancer

RESEARCH SUPPORT:

(1) Ongoing Research Support
American Cancer Society (ACS) RSG-09-082-01-MGO   (PI: Sui)   01/01/2009 – 12/31/2012
Research Scholar Grant $177,083 (/year)
Title: The role of YY1 in prostate cancer
Role: PI   26% efforts
The overall goal of this project is to study (1) the correlation between YY1 expression and prostate cancer formation, (2) the effects of YY1 increase/decrease on prostate tumorigenesis and (3) the roles of YY1-mediated Ezh2 and p53 function in prostate cancer development.

NIH R01 (PI: Cramer)   7/1/09 - 6/30/14
National Institutes of Health (NIH) $200,000 (/year)
Tak1, A novel prostate cancer tumor suppressor
This project is to determine whether Tak1 is a new tumor suppressor in prostate tumorigenesis using a mouse model.
Role: co-PI 3% efforts

NIH R01 (PI: Inoue)   7/1/09 - 6/30/14
National Institutes of Health (NIH) $200,000 (/year)
Dmp1-p53/YY1 interplays and their roles in lung cancer development
This project is to determine whether the interplay among Dmp1, p53 and YY1 is essential to the development of lung cancer.
Role: co-PI 7.6% efforts

(2) Research Completed in the Past Three Years
Golfers Against Cancer (GAC) (PI: Sui) $35,000 (/year) 04/01/2008 – 03/31/2009
Title: The Prognostic and Therapeutic Target Potential of YY1 in Breast Cancer
The goal of this project is to determine whether YY1 is a prognostic marker and therapeutic target of breast cancer.

Department of Defense (DOD)   PC060490   (PI: Sui)   03/01/2007 – 02/28/2010
New Investigator Award $225,000 (total direct cost)
Title: The role of YY1 in prostate cancer
Role: PI   20% efforts

The overall goal of this project is to study whether YY1 alteration can affect prostate cancer formation.

SERVICE IN REVIEW COMMITTEES:
2008   DOD Endocrinology (END) peer review panel of Prostate Cancer Research Program (PCRP)
2009   DOD Clinical Health Science and Epidemiology (CHS/EPI) online pre-proposal review panel of Prostate Cancer Research Program (PCRP)
2010   DOD Genetic Cancer (GC) peer review panel
2011   DOD Clinical Health Science and Epidemiology (CHS/EPI) online pre-proposal review panel of Prostate Cancer Research Program (PCRP)

GRADUATE STUDENTS/RESIDENTS/FELLOWS ADVISED:
Graduate students:
Paul Cao, since 09/2005
Weiwei Huang (visiting student), since 10/2008
Dana Yancey (rotation)
Raul Puente (rotation)
Yufeng Song (rotation)
Kristen Stadelman (rotation)
Caroline Schnegg (rotation)
Ryan Thys (rotation)
Daniel Stovall (rotation)

Postdoctoral fellow:
Zhiyong Deng (01/2006 - 08/2008)
Qiang Zhang (01/2010 - …)

THESIS COMMITTEES OF GRADUATE STUDENTS:
Paul Cao
Min Wu
Sofia Maund
Carol Chunmei Xie
Andrew Burke
Shuai Tang
Yufeng Song
Dana Yancey
Dejan Maglic
Janel Suburu